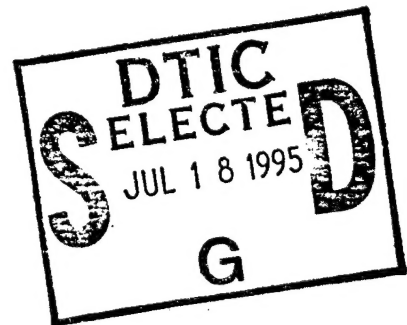

QUARTERLY PROGRESS REPORT

RESEARCH ON NAVY-RELATED COMBAT
CASUALTY CARE ISSUES, NAVY
OPERATIONAL-RELATED INJURIES AND
ILLNESSES AND APPROACHES TO ENHANCE
NAVY/MARINE CORPS PERSONNEL COMBAT

Prepared for

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Bethesda, Maryland 20814

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Prepared by
GEO-CENTERS, INC.
7 Wells Avenue
Newton Centre, MA 02159

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**RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES,
NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND
APPROACHES TO ENHANCED NAVY/MARINE CORPS PERSONNEL
COMBAT PERFORMANCE**

I. INTRODUCTION

This report summarizes the results of GEO-CENTERS' technical activities for the second quarter of the contractual base year for the Naval Medical Research Institute (NMRI) under Contract N00014-95-D-0048, Delivery Order #001. This delivery order encompasses a variety of scientific studies that are capable of supporting ongoing and projected programs under the cognizance of NMRI; NMRI TOX/DET-Dayton, OH; NDRI-Great Lakes, IL; the NDRI Detachment-Bethesda, MD; and the National Naval Medical Center-Bethesda, MD.

The format for these periodic technical progress reports consists of four sections each listed by the location of the research. The sections are (1) Descriptions of work to be performed, (2) Objectives planned for the current reporting period, (3) Significant results, and (4) Objectives for the next reporting period. Accumulated scientific reports, technical reports and journal articles will be provided as part of the annual technical progress reports. Specifically, the research conducted by GEO-CENTERS during this quarterly reporting period has been focused on the following general scientific programs:

1. Infectious disease threat assessment and enterics programs.
2. Toxicological studies.
3. Immune cell biology, wound repair and artificial blood studies.
4. Biomedical diving programs.



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5. Dental related diseases.
6. Personnel performance enhancement programs
7. Breast Care Center

INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

(1) GEO-CENTERS has been tasked to conduct R & D in support of the U.S. Navy Enterics program. We are investigating cytokines which are antigen-nonspecific mediators that play an important role in regulating immune responses as well as disease outcome. Dependent upon the site of production, concentration, and concomitant presence of other mediators, a given cytokine may contribute to the pathogenesis of an invasive organism like *Campylobacter jejuni*. In the present study, the role of local cytokines in infection and immunity to *C. jejuni* infection in mice is being evaluated.

(2) In order to know whether animal proteins were expressed in the retroviral-transformed cerebral microvascular endothelial cells and some cell wall-deficient microorganisms, a Western blot technique was employed. A monoclonal antibody against human serum albumin (HSA) and polyclonal antibody against protein kinase C (PKC) were used as probes.

Anti-HSA antibody is specific for human plasma albumin and recognizes the episode resistant to reduction or denaturation commonly used in immunoblotting techniques or in immunohistochemistry. Cross reactivity is observed with rhesus, baboon and gibbon albumin, but not with marmoset albumin. No cross-reactivities are observed with other animals.

PKC, a family of closely related enzymes, plays a central role in the signal transduction of many short-term responses. These include secretion of neurotransmitters, hormones and enzymes in muscle contraction and alterations in membrane ionic conductance. In addition, PKC activity has been implicated in the regulation of long-term responses, such as gene expression and cell proliferation. Thus, PKC is a very good representative of important mammalian proteins. Antibody against pan PKC can detect all the subtypes of PKC.



(3) Respiratory virus outbreaks among Navy and Marine Corps personnel have been prevalent for decades. Population densities and shipboard environment, both in and out of port, provide excellent conditions for transmission studies. One of the diseases that is being investigated by the GEO-CENTERS' staff is the Peruvian Measles Epidemic which is spreading throughout the world.

(4) GEO-CENTERS has been tasked to support the Navy's Infectious Disease Threat Assessment program. We are developing an experimental model using a mouse strain called "Severe Combined Immunodeficiency" (SCID) for dengue hemorrhagic fever/shock syndrome (DSS). The model will be used for testing vaccines and understanding immunology and pathogenesis of these diseases. The primary support for the SCID mice program has been at NMRI however, FY94/95 funding for this program has been cut significantly and GEO-CENTERS' investigative team on this program have been transferred to other critical infectious disease programs.

The Septic Shock Research Program (SSRP) is involved in studies into the causes, course, prevention and cures of sepsis and septic shock. Within the SSRP, the new Animal Physiology Branch which GEO-CENTERS is starting up focuses on studying the effects of sepsis on oxygen transport in tissues. Interest in this area stems from the fact that septic patients generally have well oxygenated blood and yet some or all of the tissues appear to become progressively hypoxic. Oxygen transport may be impaired at a number of steps between the lungs and the mitochondria. The first problem to be addressed will be the suspected degradation of the oxygen diffusing capacities of various tissues. Sepsis results in severe tissue edema and this edema may result in significant changes in tissue composition and the oxygen pathways as it moves from capillaries to the mitochondria. These changes may reduce the oxygen diffusion coefficients of the tissue as well as reducing the solubility of oxygen in the tissue. The consequent reduction in the amount of oxygen which moves through tissues means that although the blood contains sufficient oxygen it cannot leave the circulatory system quickly enough to maintain the mitochondrial supply. Initial studies are being conducted to determine which tissues are being most severely impacted by sepsis-induced edema. Other studies are being established to measure oxygen diffusion coefficients and oxygen solubilities in tissues.

Several additional studies are being established on other aspects of oxygen delivery problems during sepsis. In conjunction with researchers at the University of Pennsylvania's Nuclear Magnetic Resonance Center, we are using NMR to measure the skeletal muscle intracellular oxygen levels by observing the degree of myoglobin deoxygenation. Comparison of normal and septic animals will indicate whether the oxygen problems caused



by sepsis are located external to or within the myocytes. Another study will determine if the degree of sepsis can be established by following changes in respiratory and metabolic parameters that can be measured in the expired air. This offers the advantage of being relatively noninvasive while allowing continuous monitoring of one indicator of patient status. Finally, initial work has begun on determination of the effects of sepsis on the oxygen affinity of hemoglobin.

(5) A portion of the Navy's R&D HIV program involves work in Okinawa and Japan. GEO-CENTERS has been tasked to organize and manage the data pertinent to both the human T-cell leukemia/lymphoma virus type-I (HTLV-I) viral epidemiology project (VEP) based in Okinawa. Additionally, we are to geographically type human immunodeficiency virus isolates in Navy personnel and conduct epidemiological analyses of these data.

(6) Additional effort in support of the Rickettsial program has lead GEO-CENTERS to isolate and characterize gamma-interferon inducible genes and their products. This is being done in order to more clearly define their role in the functioning of the immune system.

(7) In addition to the above, the Navy's Infectious Disease Threat Assessment program has tasked GEO-CENTERS to characterize and analyze modified lysine in the surface proteins of *Rickettsia prowazekii* and typhi, as well as to find epitopes on the SPA (surface protein antigen) using pin technology.

TOXICOLOGICAL STUDIES

(1) The specific direction taken by our GEO-CENTERS' investigators at NMRI/TD continues to be focused on two major areas of concern for the U.S. Navy as they relate to medical concerns on board military aircraft, surface ships and submarines: (a) methylene chloride metabolism and carbon dioxide production (b) the pulmonary effects of toxic dust and smoke inhalation.

In studying the metabolism of methylene chloride for PB-PK modeling, we have been using a 9.2 liter closed circulating chamber for exposure and measuring the methylene chloride uptake and carbon monoxide production using on line gas chromatography. The drawbacks in this system are: large chamber volume, accumulation of expired CO by a single rat takes considerable time to detect the concentration, and estimation of carboxyhemoglobin (COHb) can be achieved only at the end of the exposure period. To circumvent the above mentioned drawbacks we changed the closed circulating chamber design to a nose-only



exposure system with 700 ml chamber volume with the tail projecting out of the chamber thereby allowing us to withdraw blood from tail vein for COHb and plasma CO₂ estimation at any time point during the six hour exposure.

The pulmonary effects of toxic dust and smoke inhalation research is part of the research program to evaluate pulmonary toxicity. Such toxicity results from inhalation of complex atmospheres composed of one or more toxic vapors/gases and which also have a high aerosol particle concentration. Recent events have revitalized interest in the comprehensive study of the pulmonary effects of vesicant warfare (CW) agents such as 4-bis (methylchloroethyl) sulfide, more commonly known as mustard gas (HD).

Recent reports (USAMRIID-SP-87-03, and Papirmeister et. al., 1991) show that mortality from exposure to HD is relatively low (1-2% of those exposed). Virtually all HD fatalities can be attributed to pulmonary effects and nearly all those exposed to HD (95%) develop debilitating acute respiratory complications such as acute tracheobronchitis, pneumonia, and adult respiratory distress syndrome (ARDS). Characterization of the mechanisms and pathogenesis of HD induced pulmonary injury is complicated by the fact that HD inhalation exposure can result from HD vapor alone or from the so called "dusty mustard" (dHD) preparation in which HD is delivered to the respiratory tract as a complex of HD vapor and HD adsorbed on respirable aerosol particles.

Studies have shown that pollutant gases or vapors that normally do not reach the distal part of the lung may do so when adsorbed on the surfaces of respirable particles and these complex atmospheres may produce physiological effects not induced by either agent alone (Boren, 1964, and Kilburn and McKenzie, 1978). Therefore dHD compared to HD will have a different distribution, clearance and retention in the lung which could result in a more severe pathogenesis of pulmonary injury. Potentiation of the lethality of inhaled CW agents in rodents delivered in aerosol form has been reported (Wheeler, 1946). Enhancement of the dermal toxicity of HD delivered as dHD was also reported in these early investigations.

(2) The GEO-CENTERS' toxicologists at the NMRI/Toxicology Department have been tasked with the continuation of the design and development of inhalation toxicology exposure facilities and to initiate a series of experiments to investigate the pulmonary toxicity of complex (aerosol and toxic vapor) atmospheres. Before initiation of experiments to study "The Pulmonary Effects of Toxic Dust and Smoke Inhalation: Mustard Gas and Dusty Mustard Surrogates" several analytical problems required solution. These problems were resolved, an experimental was formed, and the first in a series of 6 exposures was



successfully completed. Our investigators anticipate completing the full series of studies by the end of the next quarterly reporting period.

(3) One of the many toxicology programs supported by GEO-CENTERS' investigators at the U. S. Air Force Armstrong Laboratory for Toxicology in Dayton, Ohio is "species differentiation in skin penetration". The skin is only a partial barrier and it is of great importance to toxicology as a primary or secondary route of exposure. The structure and composition of each laboratory animal skin varies within the species and from human skin. Although descriptive studies suggest that species differences in dermal absorption are due to these physical and physiological dissimilarities, there has been no viable attempt to understand the underlying principles which are responsible for the differences. For inhalation of chemicals, physiologically-based pharmacokinetic (PBPK) modeling has proven to be a useful tool to predict blood concentrations and tissue doses in humans based on measurable physiological and biochemical differences. This research project will provide an analogous tool for species extrapolation when the skin is the route of absorption. By careful investigation of the effects of species differences in skin structure on permeability constant, the species extrapolation can be achieved and this is critical for accurate dermal risk assessments for various U.S. Air Force chemicals.

(4) GEO-CENTERS' investigators are continuing to conduct toxicological research on various toxins and their effects on human performance. Specific attention is placed on environments associated with Navy submarines, aircraft, and the contaminants found in military operations. Our efforts not only deal with a variety of animal models but are involved in developing physiologically-based pharmacokinetic models so that more realistic simulation of toxic effects can be made. In addition, we have been tasked to study the operant behavior of a variety of animal species during exposure to toxins found in military aircraft, ships, and submarines in order to determine possible performance decrements when humans are exposed to certain toxins.

(5) Two investigators at the senior scientist level were hired this quarter to support the Tri-Service Toxicology (U.S. Naval Medical Research Institute Detachment (Toxicology), U.S. Air Force Armstrong Laboratory Toxicology Division, and U.S. Army Medical Research Detachment, Walter Reed Army Institute of Research) research programs collocated at Wright-Patterson AFB, Dayton, Ohio. Dr. James N. McDougal was hired as a senior scientist to conduct research under the "species differences in skin penetration" project funded by the U.S. Air Force Office of Scientific Research. Dr. Daniel J. Caldwell was hired as a senior scientist to conduct occupational and environmental toxicology research on explosives and propellants, combustion toxicology, and the Persian Gulf



Veterans research project funded by the U.S. Army Medical Research and Materiel Command. As a GEO-CENTERS Department Manager, Dr. Caldwell manages GEO-CENTERS personnel assigned to Air Force and Army projects, and facilitates Tri-Service Toxicology project development.

IMMUNE CELL BIOLOGY, WOUND REPAIR PROGRAM AND ARTIFICIAL BLOOD STUDIES

(1) The Navy's Immune Cell Biology program (ICBP) has continued to task GEO-CENTERS to support the program with expertise in cellular immunology and immunopathology by investigating and developing various forms of immunotherapy for the treatment of infections and the development of hematopoietic reconstitution strategies.

(2) During Operation Desert Storm, the need for rapid detection of biological warfare and infectious agents became apparent. Devices which were commercially produced for the armed services failed to meet necessary requirements. The greatest problem with the use of these devices was the high rate of false positives. The mission of the laboratory is development of rapid (minutes) detection devices for use by U.S. forces anywhere in the world. Preliminary results from these devices indicate that present designs can produce results as sensitive as current ELISA techniques only faster. The design would be easy to use in the field and is based on state of the art immunochemistry reactions using latex beads imbedded on flow-through membranes. Development is performed under the FDA's Good Laboratory Practices (GLP's) since the final product will be considered a diagnostic device.

(3) The U.S. military is most interested in developing a means to better understand and accelerate the time it takes for a wound to heal. The more complete and fast the process the more lives of soldiers and sailors will be saved. GEO-CENTERS' investigators are actively participating in wound healing and repair studies in conjunction with NMRI scientists.

(4) The U.S. Navy R&D program studying cytokine receptors has tasked GEO-CENTERS to study the differential expression of cytokine receptors and/or p30-like genes in a cerebral microvascular endothelial cell (PCMV) self-organization system.



DENTAL RELATED DISEASES PROGRAM

(1) GEO-CENTERS has been asked to assist with a major problem in the clinical practice of periodontology. Historically, the greatest oral hygiene problem for military personnel is a variety of periodontal diseases. GEO-CENTERS' primary task is to improve early detection of the diseases and differential diagnosis of individual periodontal diseases by using immunodiagnostics.

Immunodiagnostics require specific antibodies, several of which exist at NDRI. Various polyvalent and monoclonal antibody reagents have been developed for use in immunodiagnostics directed towards *T. denticola* sp. and *P. gingivalis* epitopes using whole cell antigens or outer surface antigens. In addition to specific antigens, specific proteolytic activities have been reported, the so-called 'trypsin-like' activity that may be associated with these pathogens. Nucleic acid based probe diagnostics require the development of specific sequences unique to the desired target. Potential targets may be short sequences derived from these proteolytic genes, from variable regions of the 16S rRNA, or from plasmids isolated from targeted species.

(2) As part of our continuing R & D support for the Naval Dental School (NDS), which is a Detachment of the Naval Dental Research Institute, GEO-CENTERS' has been tasked to help establish and maintain a microbiological/immunological research program and to develop cooperative research projects between the NDS and other dental R & D organizations both within and outside the Navy.

BIOMEDICAL DIVING PROGRAMS

(1) GEO-CENTERS has been tasked to support the Navy diving biomedical program by studying the use of hydrogen as a component of breathing gas in deep dives. Specific areas of interest include development and certification of the new Phase III Hydrogen Training Facility. This will include the manufacture of a sophisticated hydrogen-supported hyperbaric chamber.

(2) In addition to support for the diving program, we are formulating and conducting studies to examine thermal balance in a hyperbaric environment and to define physiological events that occur during rest and exercise in a thermally stressful environment.



(3) GEO-CENTERS is actively studying the effects of high pressure associated with Navy deep diving operations on the neurological system of the body. Accordingly, our investigators are conducting hyperbaric research testing various diving depths using mixed gases. The effects on the nervous system of various animals is being conducted prior to the use of human subjects.

(4) GEO-CENTERS has been actively developing diving schedules and recompression schedules via modeling techniques using known algorithms.

PERSONNEL PERFORMANCE ENHANCEMENT PROGRAMS

(1) In support of the Navy/Marine Corps personnel thermal studies, GEO-CENTERS has continued to conduct R & D in thermal science with respect to physiological performance and operational medicine. These studies are in support of microclimate cooling of shipboard damage control/firefighting personnel.

(2) In our continuing support of the U.S. Navy Submarine Medical Research programs, GEO-CENTERS has been tasked to develop computer programs for the submarine medical vision effort. This includes a variety of computer environments used in reaction time-based psychological experiments, whose goal is to enhance the performance of submarine crew members. In addition, our investigators have been tasked to improve the target recognition and identification for submarine sonar crews through improvement of the visual and auditory components of sonar signals and monitors.

(3) The Navy's Special Warfare (SPECWAR) program has tasked GEO-CENTERS to evaluate effectiveness of laboratory and field training program(s) of SPECWAR personnel to recommend future guidelines to optimize performance, minimize injury, and define and diagnose musculoskeletal injuries during physical conditioning of SPECWAR trainees.

With the increased potential for low-level conflict replacing the threat of large scale warfare, there will be an increasing reliance on special operations during military operations. The training of special forces personnel is both expensive and extensive thus limiting the manpower available. These individuals must be capable of performing a wide variety of covert operations in a large number of operational environments. It is therefore critical to minimize individual performance degradation which may occur during military operations requiring the use of special forces.



BREAST DISEASE CENTER

(1) GEO-CENTERS has been tasked by NMRI to support their newly designated program to improve the Navy's understanding of breast diseases (Breast Care Center) (BCC), with a specific focus on breast cancer. The specific tasking to GEO-CENTERS is to develop the staffing plan for the R&D specialties. This includes identifying the programs' research subjects, screening the subjects for related medical history, facilitating the clinical protocol, collecting and analyzing the data, and preparing the findings for publication or presentation. It is anticipated that about 20-21 total personnel will be hired to staff the BCC's personnel needs.

(2) The radiology portion of the staffing needs consisted of three personnel who were hired and began working in June 1995. They will support the BCC mammography efforts in the Radiology Department of the NNMC.

(3) Another 12 personnel have been or will shortly be hired to begin working by September 95 with the remaining 5-6 personnel to be on board by 1 October 95.

(4) Until the BCC officially opens for business (estimated 30 August 1995) the next Quarterly Progress Report will focus only on the progress of staffing the Center. A more complete personnel and research status report will appear two quarterly technical reports from the date of this one.



II. NMRI, Bethesda, MD

A. INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

DESCRIPTION OF WORK TO BE PERFORMED

Jendrek

Mr. Jendrek conducts BL-3 fermentations and for some organisms but may also perform some or all aspects of purification associated with the project. He creates documentation including Standard Operating Procedures, Batch Records, and any documentation required for newly installed equipment. Mr. Jendrek also either installs new equipment related to the project or oversees their installation by technicians sent by the manufacture. He also assists in the molecular biology aspects, DNA purification, plasmid isolation, electroporation, and other techniques performed on a regular basis.

Weeks

Serves as an associate of the principal investigator for a research program involving pathogenic, molecular, and biochemical analysis of bacteria and their virulence factors. Experimentation requires a knowledge and proficiency of laboratory techniques and procedures for performing biochemical and immunological analyses. Conducts surveys of the scientific literature to develop background data on techniques and formulate approaches for the investigations, develops experimental protocols, defines the objectives and priorities of subsidiary problems, and arranges details of cooperative investigations with other organizations when necessary. Performs immunological techniques for quantifying microbial antigens and host cellular responses by employing appropriate molecular biochemical and immunochemical techniques. Identifies and analyzes microbial constituents and products associated with host responses to infection. Is responsible for the general preparation of laboratory reagents, solutions, enzymes, and other materials and equipment used to conduct the studies described. Is responsible for the cleanliness and orderliness of working areas, freezers and refrigerators. Is responsible for training and orientation of all new laboratory technicians. Organizes and accumulates repositories of bacterial strains, plasmids, enzymes and sera with sufficient documentation. Maintains sufficient stocks of all reagents, supplies, and equipment required for a well organized molecular biology laboratory. Performs other duties as assigned. Immunizations are required.



TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Jendrek

This quarter Mr. Jendrek will update his batch record from final draft to final copy. He will then perform more fermentations using this protocol for the monkey trials and for general research use. He will again need to repeat a fermentation for toxinology of *Pichia pastoris* BOT-E. Scott will also perform a fermentation using a PA producing strain of *B. subtilis* for comparison studies and purify the PA produced by this strain. Scott will install a new centrifuge for harvesting of the 20 liter fermentor and create documentation for use of this equipment. He will also start on a batch record for fermentation of *B. anthracis* in the 20 liter fermentor.

Weeks

The objective this quarter is to map the largest plasmid in *Yersinia pestis*, the pFra plasmid, by using mutant strains of the plasmid. This is to be accomplished by making plasmid preparations of the recombinant strain called CO92, Pst (-) and running cesium chloride purification gradients to isolate the plasmid without chromosomal contamination.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Jendrek

This quarter Mr. Jendrek updated the 5 liter batch record to final copy and created a 20 liter batch record as well, which should be ready for final copy in the early part of next quarter. He performed two fermentations for Toxinology of *Pichia pastoris*, both of which were successful in the utilization of methanol. Mr. Jendrek also performed fermentations of Delta Sterne pPA102(CR4) strain. The new centrifuge is up and running with set-up SOP incorporated into the 20 liter batch record. So far it works very well. He has also performed most of the HPLC buffer prep and clean up of columns. The PA producing strain of *B. subtilis* was not fermented due to time constraints and altering of this project.



Weeks

The mapping of the above mentioned plasmid has been slow to materialize because this plasmid is a very low copy plasmid due to its size (100 kilobases). Because of this, most of the quarter has been spent making large scale plasmid preparations of the CO92, Pst (-) recombinant strain. During the beginning and middle of the quarter, work was continued on the construction of a pFra plasmid library from the above mentioned recombinant strain. The quarter was also spent training the new Army technician who started work in the laboratory.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Jendrek

Next quarter Mr. Jendrek will perform more fermentations of Delta Sterne pPA102(CR4) to stock pile the protein for future research and trials. The 20 liter fermentations will also be repeated to show reproducibility and to test the batch record for that process. Work with mutants of the PA gene will need to be grown and purified to test immune response in Guinea pigs. Other objectives are not yet known.

Weeks

The objectives for next quarter are to continue the mapping of the pFra plasmid, continue the construction of the pFra library, and to continue training the new Army technician in the laboratory.



II. NMRI, Bethesda, MD

**B. IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND
ARTIFICIAL BLOOD PROGRAM**

DESCRIPTION OF WORK TO BE PERFORMED

Artificial Blood Research Group

Mincheff

As a senior scientist, Dr. Mincheff assists in the design and planning of experiments necessary to carry out the objectives of the artificial blood research group. He independently plans the details of each bench experiment, executes the research with the assistance of technicians, and analyzes the data for presentation and publication as appropriate.

Hammett

As a research technician, Mr. Hammett independently carries out or assists in experiments directed at meeting the research objectives. He analyzes data from each experiment for discussion with principal investigators, maintaining all necessary laboratory records. As needed, he maintains laboratory supplies and equipment for performing the assigned task experiments.

Mesbah-Karimi

As a research technician, Ms. Mesbah-Karimi independently carries out or assists in carrying out experiments designed to meet the required research objectives. She analyzes data from each experiment carried out for discussion with principal investigators, maintaining all necessary laboratory records. As needed, she maintains laboratory supplies and equipment for performing the required research tasks.

Trickett

As a research support assistant, Ms. Trickett is responsible for ensuring that the laboratories performing the assigned research have all the necessary support, supplies, and equipment needed by investigators and technicians performing the assigned research tasks. Any PC



software and editorial support needed by the research teams to meet the task objectives are the responsibility of Ms. Trickett as well.

Tsonev

As a senior scientist, Dr. Tsonev, together with other investigators, assists in the design and planning of experiments necessary to carry out the objectives of the research task. He independently plans the details of each bench experiment, executes the research and analyzes the data for presentation and publication as may be appropriate.

Okouchi

As a senior scientist, Dr. Okouchi, together with other investigators, assists in the design and planning of experiments necessary to carry out the research objectives. He independently plans the details of each bench experiment, executes the research and analyzes the data for presentation and publication as appropriate.

Li

Following moderate to severe hemorrhagic shock (HS), enteric bacteria may invariably transfer from the bowel to the systemic circulation, producing sepsis and severe complications. While adequate fluid replacement may alleviate the immediately life-threatening symptoms, loss of the enteric barrier integrity may lead to septic complications, multiple organ failure and death. Profound immunosuppression has been seen following HS. Dr. Li found that oral treatment with supernatant from Con A activated spleen cells, containing various crude cytokines, markedly reduced the number of enteric bacterial colonies. Both cellular and humoral immune response were elevated in these mice. Her results suggest that even crude cytokines may play a significant role in the amelioration of bacterial translocation, either by augmenting the immune responses or by altering the enteric barrier. Using a modified murine HS model, the oral administration of cytokines to prevent bacterial translocation from the gut to the systemic circulation has been studied.

Lipopolysaccharide (LPS) from gram-negative bacteria are considered to be the responsible agents for induction of endotoxic shock, affecting the liver and intestine as their target organs. Relevant cell lines have been selected and these cells will be incubated with LPS in anaerobic condition to mimic HS *in vitro*.



Fan

Junior Investigator, Septic Shock Research program. Specialized in molecular biology technology.

Chavez

At the Blood Research Detachment of Walter Reed Army Institute of Research (WRAIR), Mr. Chavez studies aspects of blood research. This includes basic research on the physical properties of hemoglobin, red cell storage and preservation, blood banking, and multiple levels of clinical trials on hemoglobin-based blood substitutes. His role at WRAIR involves basic research on the properties of hemoglobin and hemoglobin-based blood substitutes and technical assistance for pilot plant operations. Hemoglobin is the protein responsible for oxygen transport. Hemoglobin oxidation, toxicity, and nitric oxide binding are some of the current problems in the field of hemoglobin-based blood substitutes. He has designed several projects in order to more fully understand the mechanisms involved in hemoglobin function. This knowledge will hopefully allow for alleviation or elimination of problems associated with hemoglobin-based blood substitutes and lead to a viable blood substitute product.

Ring

The function of this position has been to provide technical support for biological investigators in the Immune Cell Biology Program.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Artificial Blood Group

The goal for this NMRI-based research team is to achieve an understanding of the mechanism by which the American Red Cross (ARC) red cell storage solutions extend the shelf-life of red blood cells stored at 4°C, and thereby suggest ways storage shelf-life times can be further increased to improve blood supply logistics.

Specifically, the objectives of the program will be to: (1) investigate the mechanism by which the depletion of chloride improves the storage characteristics of red blood cells independent of pH by altering the rates of production and consumption of ATP and 2,3-DPG;



(2) study the red cell energy budget by testing the effects of actions that may mimic chloride depletion effect; and (3) determine the effect of pH during storage and its relationship to the chloride depletion effect.

Li

- Use a murine HS model to study the effect of the oral administration of IL-6 on bacteria translocation and immune response.
- In order to investigate the intestinal vasodilation function after IL-6 administration, it is planned to establish a rat intestinal vascular perfusion model.

Fan

Continue the investigation of gene expression of inducible nitric oxide synthase (iNOS) using reverse transcriptase-polymerase chain reaction (RT-PCR) and Southern blot techniques. This is a major mediator of reduction of cardiovascular muscle contractility during sepsis and endotoxemia.

Investigate the effect of LPS on PKC mRNA transcription and identify the 8 distinct PKC isotypes by RT-PCR and Southern blot, eventually sequencing of the PCR products.

Set up a non-radioisotopic DNA sequencing system in order to analyze the PCR products of PKC mRNA.

Use constructive mimic DNA to develop a competitive PCR technique to measure iNOS mRNA quantitatively.

Chavez

With the pilot plant in full operation, the majority of attention was dedicated to research projects. The preparation of a manuscript involving near-infrared spectroscopy was a top priority. In addition, completion of the heme association experiments and initiation of heme exchange experiments are expected to be accomplished.

During the next six-to-nine months, another cross linked derivative will be produce in the pilot plant for research purposes. Cross linked hemoglobin at b82-82b inhibits the tetramer ->dimer equilibrium similar to the a99-99a hemoglobin derivative currently being produced. However, the b82-82b derivative exhibits a higher oxygen affinity. Comparative studies of



the two cross linked hemoglobin derivatives will yield insight into the capacity for oxygen delivery for the derivatives.

Ring

Biological research, as in other areas, involves computers for many activities: data organization communication, etc. However, many of the investigators are not trained in these disciplines.

GEO-CENTERS' presence within the Tissue Bank gives the biological investigators a source of help in resolving technical problems. The objectives for the quarter are not hard scientific accomplishments, but rather support functions which contribute to the overall operation of the Immune Cell Biology Program.

The first objective is to provide general computer support. Support for stand-alone computers involves assistance with spreadsheets, databases, and operating systems. Support for network connectivity involves hardware installations, software configuration, and personnel training.

The second objective is to maintain the availability of I.C.B.P.'s calcium imaging system. The system is built around a MicroVax II main processor with an attached Gould image processor. Software from R.Y. Tsien (University of California) was used as the software base. Numerous hardware and software enhancements have been done. The result is a system highly specialized for the measurements of intracellular calcium. Use of the system has fluctuated, with active periods and slack periods. The objective is to manage the system during the active periods and maintain a "corporate memory" during the slack periods.

The general aim of work has been and continues to be: how can the tools of computing resources be made available to the biological investigator? In imaging, networks, and programming, the objective is to be responsive to the needs of individual investigators and the department as a whole.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Artificial Blood Group (NMRI)

Specific progress in the second full quarterly report is described below.

Immunology Project - This project is directed at acquiring an understanding of the effect of residual leukocytes on the storage and subsequent transfusion of red cells.

It has been found that, after the storage of washed red cells for six weeks, despite excellent morphologies and chemistries, *in vivo* survival is quite poor. Filtration of the red cells to remove leukocytes prior to washing greatly improves 24-hr survival.

We have also shown that when red cells are washed, the associated depletion of plasma drives all leukocytes into apoptosis. Associated with apoptosis is the expression of membrane surface markers, particularly phosphatidyl serine, that signal for phagocytosis. Where as *in vivo*, apoptosis thus results in phagocytosis, apoptotic cell form blebs, lyse and disintegrate. It has been proposed that it is the absorption onto otherwise normal red cells that leads to unexpected disappearance of red cells after transfusion.

During the past quarter efforts have focused on demonstration of the presence of phosphatidyl choline (PS) on washed stored red cells. We have been successful in this effort, finding a significant difference in signal between filtered and unfiltered cells. Furthermore, we have shown that a period of incubation at room temperature or higher is necessary for the acquisition of the PS signal by red cells, suggesting that, rather than a simple adsorption of PS on to the red cell, we are seeing fusion of vesicles released by the apoptotic leukocytes.

The question whether this is a significant source of post-transfusion cell losses after the storage of unwashed red cells remains to be answered and studies to answer this question will commence during the next quarter.

A second question concerns the extent of leukocytes removal necessary to prevent cell loss by post-transfusion phagocytosis. The survival studies showing a reduction in losses with filtered cells were conducted with a second generation filter, the Pall RC300, which reduces leukocytes to about 500,000 per unit. Despite the improved survival, it still was lower than would be predicted by morphologies and chemistries. Studies will be initiated during the



next quarter to compare 24-hr survivals of washed cells filtered with the RC300 or with an experimental filter, the Pall RCZL, which reduces residual leukocyte count to near zero.

Red Cell Project - Applied and development studies of liquid stored red cells have focused on two procedures, a new additive solution and rejuvenation of stored outdated red cells.

The new additive solution consists only of glucose, phosphate and adenine. Since the glucose enters the cells slowly, osmotic support is provided during storage but, as the glucose reaches equilibrium across the cell membrane, the effective osmolality of the extracellular solution falls. This approach provides a sufficiently hypotonic medium for red cell storage that provide real benefits as compared to previous solutions containing citrate. Morphological and chemical assays show an approximately 10% elevation at six weeks compared to conventional licensed products. Survival studies are planned for the next quarter. A patent application has been filed.

Our ARC solutions serve as rejuvenating solutions, improving morphology and ATP levels, but with the advantage over existing solution of being transfusable and not requiring washing prior to use. We have devised a simple procedure for carrying out the resuspension of cells in the rejuvenation solution and a patent application has been filed. Survival studies are scheduled during the next quarter.

Basic Research Project - Studies have continued on the newly discovered physics of cold denaturation of proteins using primarily the fluorescence scanning method augmented by calorimetry and circular dichroism. Additional model proteins have been investigated and shown to have a significant destabilization transition at 0°C. This destabilization has been shown to persist for days following rewarming to physiological temperatures. As a result of this observation, a general applicability of the fluorescence ratio scanning method to the industrial processing of proteins, cryopreservation of human tissues, and hypothermic treatment of cancer was perceived. A patent application on this method was filed in May and included a novel spectrofluorimeter designed to analyze the configuration of proteins even in frozen solutions.

Continuing analysis of the most recent result has lead to a refinement of models of protein unfolding. Comparison of circular dichroic spectra to fluorescence data indicates that the random coil configuration of the unfolded proteins develop a more beta turn and alpha helical structure as the protein is cooled. These transitions to more structure appears to occur in discrete jumps and can apparently occur in both native in denatured protein states leading to a complex series of denaturation-renaturation transitions during cooling and rewarming.



Tissue Preservation Project - The current quarter has been devoted to reestablishing the techniques necessary for the perfusion and transplantation of rat liver. It has been necessary to modify a section of the laboratory and refurbish several pieces of equipment. The transplantation technique is now working smoothly. Transplantation survival following introduction and removal of a cryoprotectant perfusion solution, a DMSO-propylene glycolformamide mixture, has yet to be achieved.

Li

We have worked on HS model continuously fed with IL-6 media. After exposure to HS for 16 hrs the proliferation of bacteria in liver and the spleen cell have been measured.

Rat mesenteric artery perfusion method: Rats were anesthetized with pentobarbital and 100u was injected through the tail vein. The superior mesenteric artery was cannulated with PE 50 and perfused with Krebs-Henseleit buffer. The liquid which flowed through the cannulated port vein was harvested and analyzed.

Immunocytochemical staining of the P388 D1 (mouse macrophage), BNL, BNL Mea (mouse liver cells), HCT-8, HTB-40 (human intestine epithelia cells) were performed using rat anti mouse IL-6 and rabbit anti human IL-6 antibodies.

Frozen sections and paraffin sections of the mouse intestine were immunostaining with rat anti-mouse IL-6 receptor antibody to study the IL-6 receptor levels of these samples.

Agarose blocks contained normal mouse pancreas islets were transplantation to diabetic mice and incubation of these agarose blocks with glucose media was done to investigate whether the transplanted block still contains the insulin bioactivity.

Significant Results:

- The number of liver bacteria was remarkable decreased and the spleen cell proliferation was elevated after the HS mice had been fed with IL-6 300u and 30u.
- In all the treated cell lines, except BNL cell (LPS 10ug/ml 4hrs), stronger immunoreactivities have been observed.
- An increased of IL-6 receptor immunostaining has been found in HS mice intestine especially in the ileum.



Fan

Generated satisfactory results on the pathways of activation of iNOS mRNA expression in rat cardiac myocytes and aorta smooth muscle cells using RT-PCR and Southern blot. Data was presented in weekly program meetings.

Generated satisfactory results on PKC (8 isoforms) gene regulation in cardiac myocytes and aorta smooth muscle cells stimulated with LPS. Data was presented in weekly program meetings.

Primarily developed the non-isotopic DNA sequencing system to directly sequence the PCR product of iNOS.

Primarily developed the competitive PCR technique using constructive mimic DNA.

Chavez

The hydrolysis of ATP utilizing $\text{Na}^+ \text{K}^+$ ATPase can be monitored using near infrared spectroscopy. Comparative studies were accomplished using an inorganic phosphate assay as the standard method of analysis and near infrared spectroscopy as a potentially new analytical method of following enzyme kinetics. A high degree of correlation was found in comparing the inorganic phosphate assay with the near infrared spectroscopy. Hence, near infrared spectroscopy was found to be quite adept in monitoring enzyme kinetics. The advantages of near infrared spectroscopy include the following: (1) low light scattering, (2) *in vivo* capability, (3) high sensitivity, (4) no exposure to radioisotopes, (5) separation of components to reveal intermediates, and (6) fast temporal resolution (<1 sec). The manuscript is being completed and submission should take place in July.

The heme association experiments have been extended. Hemoglobin contains four protein chains, each enclosing a heme active site where the oxygen binds. To preserve functionality and chains, each enclosing a heme active site where the oxygen binds. To preserve functionality and prevent toxic effects, it is imperative that hemoglobin-based blood substitutes maintain the incorporation of heme within the protein. By using rapid scanning spectrophotometry, one can directly measure the hemoglobin's affinity for heme. By comparing several varieties of heme containing globin, the mechanism by which hemoglobin retains the heme can be found. With genetic engineering and/or chemical modification of the hemoglobin, the optimization of heme retention can be understood, monitored, preserved, and potentially enhanced.



In order to perform these experiments, the rapid scanning monochromator was upgraded to incorporate stainless steel tubing. This modification establishes anaerobic conditions necessary to maintain the heme at desired oxidation states. In addition, it was determined that one more cross-linked derivative should be studied to verify the effects of internally cross linking the hemoglobin tetramer. b82-82b hemoglobin was chosen for several reasons. Currently, the same cross linker required to make a99-99a hemoglobin is used. The preparation of this hemoglobin derivative is similar to the a99-99a hemoglobin derivative we currently study, and in the near future we will be producing b82-82b hemoglobin in the pilot plan for comparative physiological studies. Previous work on b82-82b hemoglobin has shown that in reacting DBBF (the cross linker) with liganded hemoglobin, two products are formed, both of which are cross linked in the vicinity of the b82-82b binding site. However, these products have extremely different oxygen binding characteristics as shown in the oxygen equilibrium data. Further purification will be required for our heme association experiments since we have already shown that heme association is sensitive to protein structure.

Heme exchange experiments have been initiated. Heme loss can be measured using albumin as the receptor site and oxidized hemoglobin as the donor of heme. When this reaction is run at pH 9.0, large spectral changes are observed when the heme releases from the hemoglobin and binds to the albumin. Hence, heme loss can be easily measured. Preparation of partially oxidized samples will determine to what extent protein configuration will affect the rate of hem loss.

Ring

- The mice database program was upgraded. There are now two versions of the program Dr. R. Abe's group analyzes blood types and uses the first version. The second version was written for Dr. David Harlan's group which analyzes a different set of criteria. The program was redesigned to allow for flexibility in genealogical hierarchies and testing criteria.
- Assistance was provided on spreadsheets and databases for use by I.C.B.P.'s administrative personnel.
- General support was provided in the department's use of NMRI's network. Training continued in investigators' use of the network.
- General support was provided in assessing and organizing the department's use of commercial software packages.
- Support was provided to run the calcium imaging system for visiting NIH personnel.



GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Li

Repeat the IL-6 dose dependent curve in the HS model.

Repeat the receptor immunostaining in the HS model intestine.

Continue to study the rat intestine perfusion model in order to investigate the IL-6 intestinal vasodilation function.

Fan

Continue the ongoing project on iNOS mRNA regulation pathways in rat cardiac myocytes and aortic smooth muscle cells during sepsis and endotoxemia.

Using competitive PCR technique to quantitate iNOS mRNA in rat vascular micro vessels.

Continue the project on non-isotopic sequencing of the PCR product of iNOS.

Chavez

Two summer students will work in the laboratory during the next two months. These students are participating in a summer internship jointly sponsored by WRAIR and George Washington University. One student will work on the heme exchange experiment and the other will initiate a stopped-flow experiment to investigate intermediates formed upon ligand binding to hemoglobin. In addition to supervising these students, completion of the heme association experiments and the near infrared manuscript hopefully will be accomplished.

Ring

- Develop, improve and upgrade software programs as needed.
- Assist in managing the department's use of the network.
- Continue to work with the NIH investigators in calcium studies.



II. NMRI, Bethesda, MD

C. BIOMEDICAL DIVING RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Shea

Senior Scientist II at NMRI responsible for analysis of neurotransmitters in the CNS recovered by microdialysis. This method, when used in various animal models of stress, provides samples in which different transmitters can be monitored in a living animal over time. A variety of different procedures using high performance liquid chromatography (HPLC) are used in the analysis of these transmitters. It is Mr. Shea's responsibility to establish these methods and maintain them for the above analyses. This position also requires the training of new personnel in the techniques of HPLC and microdialysis.

Obowa

Ms. Obowa supports a grant funding Diving Medicine research investigating exposure to hyperbaric oxygen (HBO) and its effects on the CNS. The purpose of this research is to identify which brain regions are most affected by HBO exposure, which regions are involved in HBO convulsions, and which neurochemical processes in these regions contribute to the occurrence of HBO convulsions. She performs technical and support work in the laboratory.

Porter

Mr. Porter supports the selection of a hyperbaric CO2 analyzer for fleet submarine dry deck shelter use and selection of an ambient air CO2 analyzer for fleet submarine use. Mr. Porter also supports analysis of fleet soda lime for possible contamination and analyzes samples for specific dye concentrations.

Ruby

Mr. Ruby provides gas analysis support for Navy diving studies at NMRI and development of new gas analysis methods in support of Navy Fleet requirements. He also supports specification, procurement and installation of laboratory chemical analytical instrumentation to support NMRI/DBTFA gas analysis capabilities.



TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Shea

We are continuing the research efforts on an animal model of Alzheimer's disease that was discussed last quarter. In the last working quarter beta amyloid precursor protein (B-APP) was induced in the cortex of rats by injections of the drug NMDA into the nucleus basalis Mynert (NBM). The contralateral side of the cortex is being used as control for drug effects. Extracellular levels of the neurotransmitter acetylcholine (ACh) have previously been reported to correlate inversely with the levels of β -APP when the NBM receives lidocaine, and microdialysis is performed ipsilaterally in the cortex. Both a sham injection will be done on the non-lesioned side as well as microdialysis in the contralateral cortex. Using this system examination of the length of time for the lesion to reduce the levels of ACh to at least 40% of the contralateral side will be studied. This will represent a complete lesioning of the ACh input to the cortex from this tract by injections of NMDA.

Continue preparations for a hyperbaric chamber to be used for microdialysis under 100% oxygen atmosphere.

Continue examining the various methods available for measuring nitric oxide.

Obowa

During this quarter, basic procedures have been learned including ordering supplies, handling and care of animals, performing surgery to implant EEG electrodes into animals, sectioning tissues using the cryostat, and staining tissue sections using immunohistochemistry to detect c-fos.

Porter

- To continue with fleet soda lime analysis as samples come in from the manufacture.
- To continue the testing program for the candidate CO2 analyzers.

Ruby

- Assist the Naval Submarine Medical Research Lab (NSMRL) in the selection, acquisition and testing of a portable carbon dioxide analyzer for use in a disabled submarine scenario.



- Assist the Naval Sea Systems Command (NAVSEA) in the development of a portable carbon dioxide analyzer capable of operation in hyperbaric atmospheres for use in Dry Deck Shelters (DDS) and host ships.
- Provide technical support to the clean van, the MRCC, and the gas farm operations.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Shea

A large number of animals have been run through the NMDA lesion protocol, with the primary variable being time post-lesion. The length of time has been extended to 72 hours. When the data for ACh was computed it was observed that it takes at least 12 hrs for a significant decrease in ACh levels to occur and that a 50% decrease was found from 24 hrs to 72 hrs. During this same time period, norepinephrine and serotonin levels remain unchanged. These observations have been submitted for a scientific presentation to the society for neuroscience held this November. Cerebral spinal fluid was also taken from each of these animals and sent to our collaborators for measurement of amyloid.

The chamber for hyperbaric studies is near completion. The instrumentation has also been modified and is ready for use. The room however is still being prepared for use and is not expected to be finished until the end of Summer.

Two companies have been contacted about their instruments for measuring nitric oxide. An electrochemical package has been ordered and is being tested that should allow doing these measurements *in vivo*.

Obowa

The objectives for the next quarter include the following:

- Get certified to use the dive chamber in HBO exposures
- Implant EEG electrodes in more animals to continue recording brain activity during HBO exposure and convulsions
- Develop immunohistochemical staining procedure for heat shock proteins (hsp)



- Perform experiments to produce convulsions using maximal electoshock (MES) and pentylenetetrazol (PTZ) and compare c-fos distribution in these cases to animals exposed to HBO

Porter

- Short term testing program has been completed for all five candidate CO2 analyzers.
- Interference testing has been completed for all five candidate CO2 analyzers.
- Long term test and temperature interference test have been completed for two of the candidate CO2 analyzers.
- Long term tests are now being conducted on the three remaining candidate analyzers.
- Hyperbaric testing of two prototype CO2 analyzers for dry deck shelter use is under way. Modifications are being made as required and the units are being retested.
- Work was started on a poster presentation "Decompression Comparison of Helium and Hydrogen in Rats" to be presented at the June meeting of the Undersea Hyperbaric Medical Society.
- Performed other laboratory as requested.

Ruby

Four candidate analyzers have been purchased and placed on long term test for the NSMRL project. Two of the analyzers have completed all of the required test protocols for this project. One has passed all of the requirements and one has failed the long term drift specification. The two remaining candidates are on track. Upon completion of testing, one candidate and one alternate will be recommended to NSMRL for final selection.

Two candidates have been selected for the NAVSEA project. Modifications have been made to both to permit their operation in hyperbaric chambers at 6 atmospheres absolute (ATA). The pre-prototype units, that incorporate the design changes required for hyperbaric use, have been received from the vendor and are undergoing the initial bank of qualification tests.

As part of this program, gas samples from the divers air banks on all of the host ships will be completely analyzed to insure that there is no interference from the divers breathing air with the analyzers under hyperbaric conditions. Six submarines have been sampled to date at Groton, CT, Norfolk, VA, and Pearl Harbor, HI.

A failure analysis investigation to determine the possibility of generating toxic gases from compressors using synthetic oils has been started as a result of a compressor failure at the



Naval Explosive Ordnance Disposal Technology Division (EOD), Indian Head, MD.

Support has been provided, as required, to other functional areas of the diving medicine department.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Shea

Continue to add more animals until we satisfy the entire time course for NMDA lesions. Test the effects of anesthetics under the conditions used in these experiments.

With the completion of the room, a study on the effects of hyperbaric oxygen in small animals will begin.

It is hoped that the type of instrument we chose will be useful for *in vivo* studies involving nitric oxide. We have hopes of also measuring free radicals under the same conditions by using a trapping agent that will be electrochemically oxidizable.

Porter

- To continue analysis of fleet soda lime for contaminants and dye concentration as needed.
- To continue testing program for candidate CO2 analyzers for both the dry deck shelter and sub ambient air programs.

Ruby

- Continue the testing of the candidate analyzers for the NSMRL project and provide periodic reporting of the progress.
- Continue the development of the NAVSEA candidate analyzers and the divers air bank sampling procedures.
- Begin a screening and selection process to obtain portable analyzers for field testing of compressors used to produce divers breathing air. Parameters will be oil mist/particulate, oxygen and carbon monoxide.
- Continue the EOD compressor failure analysis.
- Support the needs of the diving medicine functional areas as required.



II. NMRI, Bethesda, MD

D. PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Salander

Ms. Salander extended and enhanced the NMRI/Thermal Stress memory research project by contributing expertise in specific scientific areas. She also contributed to surgical procedures when needed.

Wolf

Medical Project Manager: Provided management support to the Combat Casualty Care Program at Naval Medical Research and Development Command. Duties include reviewing medical research plans and progress reports, recommending laboratory guidance, evaluating research proposals, drafting periodic and *ad hoc* management reports and developing presentation materials.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Salander

The objectives for this period were to continue an on going training program, FI/FR schedule, for new naive rats. Blood studies, designed to examine the physiologic response experienced by the rat when CRF has been administered and stress effects have been suppressed in the rat behavior, have continued to be reviewed. Rats were successfully trained to begin a study to investigate the effects on blood flow in the rat tail when a reinforced schedule has been learned and stress through shock has been introduced. Also, six rats were trained to stay in a restraining device for 20 minutes after an injection of NPY, in order to record and analyze the effects on blood flow in the rat tail.



Wolf

Want to make the transition for the new Research Area Manager (Code 45) as smooth as possible.

Complete all projects on time and with no more than 1 error per five submissions to the department director.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Salander

It has been demonstrated that CRF has been shown to influence a variety of biochemical, physiological and behavioral processes which are thought to reflect the cascade of internal events activated during acute stress. CRF causes a substantial increase in the release of central catecholamines, especially norepinephrine (NE). It is believed that CDAP will attenuate the behavioral stress effects caused by CRF. Blood studies have been added to the design of this experiment to better understand the physiologic response experienced by the rat when CRF has been administered and the stress effects have been suppressed in the rat behavior. This study is now complete and we are currently analyzing the data. Although training has been completed for the three studies noted in the "Technical Objectives" section of this report, the actual studies were delayed. The delay was related to an unfortunate cascade of events within the Thermal Stress Department and at the animal house which supplies rats for our studies. Hopefully, all situations have been resolved and the studies can now move forward.

Wolf

Met the error rate limit; actually had only one typo in the entire quarter that got to the department head.

Continued as an active participant in the interim-report review process for all submissions from all performers. It is my responsibility to monitor, communicate with Principal Investigators, resolve problems and issues, and recommend headquarters actions.



Funding Breakdown by Types of People within Internal

Thrust: Develop a spreadsheet or database such that we can identify various costs; goal is to determine where money is being spent by the performers and analyze for efficiency and effectiveness.

Status: Worked the present database to identify a start point. Made contact with various internal and external codes. Attempted to locate and use data developed for the RDA-21 study on Type I/II intramural proportions; was of minimal use. Looked into each work unit binder and extracted data from it. Using that data, created spreadsheet and worked it into a memo for the department head.

1995 Technology Area Plan (TAP) Preparation

Thrust: Review, update and complete the TAP for presentation to Senior ASBREM Officials to consolidate for ASBREM DOD (Health Affairs) & DDRE (Life Sciences) review.

Status: Worked with Dr. Feister, Major Bruttig, Dr. Majde and CAPT Hartzman. Submitted all information in a timely. While we were able to meet each deadline, the data sometimes lacked for a complete analysis; the department head was satisfied we had done all that was possible, given the moving target.

6.3A Annual Report

Thrust: Review each of the 6.3A interim reports and synopsize the accomplishments for the first half of FY95. Submit to NMRDC-21.

Status: Have done the review for all thirty-five work units and prepared the draft and final report. Submitted two days prior to suspense.

Program Review-97 R-2 Exhibit Preparation

Thrust: Review the R-2 Exhibit. Make accomplishment changes, if any, and get it smoothed and into '421 for submission to Congress via Office of Naval Research and Chief of Naval Operations by 20 April. There was a significant advancement by ONR over its original suspense date of 15 May. Guidance on format was changed multiple times here as well.

Status: Completed and submitted on time.



Foreign Comparative Testing Office Review of Australian Mobile Intensive Care Rescue Facility

Thrust: Invited to participate with the International Programs Office, Foreign Comparative Testing office in the review of a litter which has life support functions integral for use in Med-Evac vehicles.

Status: Have received initial brief. Interviewed Army, ARPA and Navy representatives who have been looking at similar devices. Made a decision to not pursue further and advised department head and prepared "no thank you letter" to FCT office.

SBIR Phase III Initiation

Thrust: Review pre-proposal, decide value, request submission of full proposal.

Status: Pre-proposal reviewed; the investigator concentrates on a new instrument, apparently oblivious to NHRC's comments on still needing large data base. Sent NHRC's summary report; then, after another review, sent letter requesting full proposal, citing the investigator's need to be keenly aware of NHRC's discussion and conclusions.

Advanced Technology Applications for Combat Casualty Care (ATACCC) Meeting - Poster Preparation

Thrust: Develop poster for ATACCC meeting.

Status: Decided what facility to do the work (GEO-CENTERS, INC.). Using department head's general concept, worked up files for the text boxes, found graphics. Shipped to GEO-Boston for preparation, received artwork back and presented at ATACCC. Graphics Artist did a really nice job! Will hang on wall in the hall.

Congressional Set Aside for University Research

Thrust: Congress sent ONR a bill to pay for its own university research initiatives. We needed to cut our university associated research to help pay that bill.

Status: Decided what to cut from which institutions (generally, it was 10% from each university). Reduction Procurement Requests were cut through ONRMIS, approved by the



Program Council and are in the works. Copies of concurrence faxes were sent to the ONR Contracting Officer.

Locate National Institutes of Health (NIH) RFP/A for Blood Substitutes

Thrust: Army representative to Blood Steering Committee stated that NIH had released an RFP/A for Blood Substitutes. Locate, read and act on it.

Status: Review of World Wide Web & Gopher Sites at NIH does not reveal such an announcement or RFP/A. Phone call with NIH "information specialist" says no current one is available, but a general announcement is available; it will be mailed to me. Further investigation on the Gopher site demonstrates an old RFA which had one paragraph about blood substitutes; it was given to department head. It was requested that I continue to search for others in which we might have an interest. This will be done.

Develop Spreadsheets for Work unit Data for each of the Research Area Managers

Thrust: Make copies of CODE 45 work unit spreadsheet for each of the Research Area Managers. Attempt to get all perspectives of the money management into one common format.

Status: Completed all but the Infectious Diseases spreadsheet and delivered to Code 04 one week prior suspense date. Discussed difficulties in trying to resolve the three sources of information and reached agreement that this would be an on-going evolution.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Salander

This will be the end of Ms. Salander's participation on these research projects since her termination of employment will be on May 19, 1995.

Wolf

NMRDC will take delivery of new personal computers, local area network hardware and all sorts of software. The major objective is to learn what the new systems will be able to do and maximize their capabilities to the benefit of Code 45.



III. NDRI, Great Lakes, IL and NDRI Detachment, Bethesda, MD

DENTAL DISEASES-RELATED RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Turner

The current research focus is directed toward specific host-defense factors which may play central roles in soft tissue disorders of concern to dentistry. Factors synthesized and released by polymorphonuclear neutrophils are of special interest. Factors found in neutrophil lysosomal organelles have been shown to have the capacity for efficient killing of microorganisms known to be pathogenic in periodontal disorders. Comparison of lysosomal activities of neutrophils isolated from peripheral blood of healthy patients with activities of neutrophils isolated from the blood of patients suffering from various forms of periodontal disorders is also being conducted.

Lamberts

To assist as an editorial consultant in the preparation or review of manuscripts to be submitted for publication. To aid in the preparation of research presentations for scientific meetings, in the review of research proposals, research communications (letters, rebuttals), etc.

Ahlf

The operational readiness of Naval and Marine personnel is the foremost mission of the Navy. In assessing the immediate dental needs relative to the mission, there are a number of supportive dental issues that need to be researched more fully and a specific plan developed. The impact of patient education, behavior modification and preventive dentistry is a concern especially in a public health environment.

Miller

Senior Research Scientist and Group Supervisor. Responsible for all aspects of immunological and microbiological activities within the Naval Dental School. This includes the development and supervision of research protocols, dental resident mentoring activities, instruction of courses in dental microbiology and dental immunology, supervision of various



GEO-CENTERS personnel, serving as a link between NIH sponsored research and Naval Dental Research programs, and troubleshooting of research programs, computers, instrumentation and equipment.

Solanki

To provide technical assistance with ongoing Immunology research projects and maintain and upgrade the laboratory environment so that research experiments are carried out smoothly. Maintains and records proper technical procedures and data produced for each experiment.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Turner

Fluorescent spectrophotometers for measurement of neutrophil biological activities have been ordered and several components of this equipment have been received, assembled and tested for working order. We still have not received several filter sets, computer equipment for operating the fluorescent spectrophotometers and some of the necessary computer software.

Lamberts

To complete revision of a manuscript for publication on immune-response indicators in saliva as required.

To complete preparation of a manuscript for Dr. Simonson on "Quantitative Relationships of Eikenella corrodens, Porphyromonas gingivalis, Treponema denticola, and Wolinella recta to the severity of localized juvenile periodontitis".

To assist NDRI investigators editorially whenever requested.

Ahlf

The appropriate paperwork for the development of an interactive dental patient education/wellness curriculum will be submitted for review on 1 June 1995. The intention is to create educational materials and video tapes directed mainly at the recruit population.



Miller

Work Unit: 0601152N.MR00001.001-0063 - Evaluation of the influence of superantigens and polyclonal B-cell activators in periodontal disease. To continue work on this Individual Research Program specifically focusing on the rt-PCR and flow work. Attempts will also be made to begin purification and characterization of superantigens and immunomodulators associated with periodontal pathogens.

Work Unit: 0601152N.MR00001.001-0063 - IL-1 production by polymorphonuclear leukocytes resident in periradicular lesions. To continue development of an *in-situ* hybridization procedure to detect cytokine mRNA.

Work Unit: 0601152N.MR00001.001-0063 - Long term frozen storage of lymphocytes. To complete the second part of this program with the quantitation of cytokines. It should be noted that enough lymphocytes remain frozen in nitrogen storage to permit us to evaluate activity for several years.

Work Unit 63706N.M0095.006-3014 - Influence of growth factors on gingival and periodontal ligament fibroblasts. A main goal will be to complete writing of the manuscript.

To initiate a new program designed to study lymphocyte heterogeneity in periradicular tissue.

Solanki

- For the study on the effects of freezing of T cells, measure various cytokine levels on supernatant of toxin stimulated cells.
- Proceed with the study "Influence of T. Denticola on cell proliferation" and start evaluating cytokine activities by ELISA on supernatant of cells exposed to various toxins.
- Continue with immunofluorescence staining experiments on lymphocytes for the flow cytometry.
- Begin operating the Millipore system for two dimensional gel electrophoresis and start separating proteins.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Turner

We have continued utilizing the SMART system for examining biochemical characteristics of neutrophil lysosomal components and several cell extracts have been subjected to ELISA using an old colorimetric systems. The ELISA will be enhanced when the fluorescent equipment is operational.

The Principal Investigator has presented data underlying progress on this Project to a Committee composed of scientists selected by the Office of Naval Research.

Lamberts

Reviewed manuscripts for Capt. D. Meyer and Lt. F. Leal.

Completed preliminary work on the manuscript of Dr. Simonson.

Completed the revision of a manuscript on "Salivary Levels of Alpha-2 Macroglobulin, Alpha-1 Anti-trypsin, C-Reactive Protein, Cathepsin-G and Elastase in Human Subjects with or without Destructive Periodontal Disease" to a "Short Communication" and addressed the reviewers' comments. The manuscript is now being resubmitted for publication.

Began work on assisting in the preparation of a manuscript for E. Pederson et al., on a new bacterial medium.

Two abstracts of the work related to this project have been presented to the American Association for Dental research and have been published in the "Journal of Dental Research" volume 74, special edition, Spring 1995.

A manuscript, based on data derived from work on this project, is now being prepared for submission to a refereed journal.

The Principal Investigator has presented data underlying progress on this project to a committee composed of scientists selected by the Office of Naval Research.



Ahlf

Accepted position of Geo Center, Inc., Great Lakes Group Supervisor.

Assisted with logistics for data collection and sample gathering at the Hines VA Hospital. This effort assisted the Scientific Department in their research of periodontal diseases.

Coordinated the tracking of patients with periodontal diseases from Clinic 1523 to Clinic 1017. Determining mechanisms necessary to track these patients after they leave Great Lakes for longitudinal study.

The Bureau of Medicine tasked NDRI to report on the periodicity of T2 examinations. Collected, photocopied and evaluated random sample of staff dental records (approximately 700) from three clinical sites at Great Lakes Naval Dental Center. Results were presented by Capt. Ralls at the Commanding Officers' meeting.

Presented *State of Union* address at NDRI's Breakfast Club meeting.

Held two part in-service at Clinic 1523 on *Safe Stress*. Questionnaire was completed and evaluated.

Submitted paperwork for privileges to treat patients non-surgically at Great Lakes Naval Hospital.

Miller

Relative to the project Evaluation of the Influence of Superantigens and Polyclonal B-cell Activators in Periodontal Disease we have utilized quantitative reverse transcriptase PCR procedures to identify superantigen production in bacterial preparations by identifying specific Vb_message production by lymphocytes exposed to bacterial superantigens. This procedure incorporates the use of a state-of-the art fluorescence gene sequencing procedure and involves a collaboration between this laboratory and the National Institutes of Health Epidemiology and Disease Prevention Branch of NIDR (Dr. Scott Diehl). Our recent work has indicated that extracts from *Actinobacillus actinomycetemcomitans* are able to stimulate expansion of T-cells carrying the TCR-Vb_1 and Vb8 markers. In addition, we have also run a number of studies to evaluate the expansion of T-cells producing various TCR-Vb__ markers on cells exposed to bacterial components using two and three parameter flow



cytometry. A presentation of this work is scheduled for the 9th International Convocation of Immunology in July.

Considerable work has also been completed concerning the influence of *Treponema denticola* on cytokine production by cells obtained from periodontally diseased and non-diseased individuals. Seven control and 6 periodontally diseased individuals have been tested and responses to *T. denticola* appear to be elevated in the diseased group. In addition, evaluation of the levels of production of IL-1, IL-2, IL-6, TNF-b, and IL-6 soluble receptor in peripheral lymphocytes from subjects with periodontitis and from non-diseased individuals after co-culturing with T.d. or various immune stimulators have been performed. We have observed that cells obtained from diseased individuals produce significantly less IL-2 when stimulated with the superantigen SEA than do non-diseased individuals. Since the superantigen TSST-1 caused similar IL-2 production in both groups, it is possible that diseased subjects had been exposed to non TCR-Vb2 superantigens. Some of this work will be presented at the International Cytokine Conference in Harrogate, UK, in September.

Relative to freeze storage of lymphocytes all bench work has been completed and manuscripts are being written.

Relative to the project "Clinical evaluation of bacterial leakage of endodontic temporary filling materials", the final the final manuscript has been written and submitted for publication.

Relative to the project "Influence of growth factors on gingival and periodontal ligament fibroblasts" we have completed all of the planned research and anticipate the completion of a manuscript by late summer.

Solanki

- All required experiments for the study of "Influence of T. Denticola on cell proliferation" were completed and quantitative proliferation data was gathered on each subject.
- For both studies, the effects of freezing of T cells and the influence of T. Denticola on cell proliferation, cytokine levels were measured using ELISA technique. IL-2, IL-6, IL-1B, and TNF-B were the specific cytokines of interests.
- The flow cytometry experiments have continuously been carried out using lymphocytes of various donors. Procedures for immunofluorescent staining for better understanding of lymphocyte activation have been improved.



- Knowledge has been gained on how to operate the Millipore 2-D gel electrophoresis system. We are now in process of separating proteins at 2-D level and staining the gel to visualize separated proteins.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Turner

We will attempt to complete the assembly of the fluorescent-associated equipment when (if) it arrives at this command. Additionally we plan to add enhanced chemiluminescent equipment for Western blotting techniques. This equipment should help us discriminate physically altered lysosomal molecules from intact molecules.

Laboratory personnel will need to familiarize themselves with this new equipment and this should require the bulk of time in the coming quarter.

Ahlf

To continue working and complete the videotape regarding tobacco.

To complete paperwork to be submitted for research approval for Interactive Dental Prevention Intervention, utilizing multi-media technology. Then begin the project using milestones.

To provide preventive and non-surgical treatment for Naval personnel at the NDRI command.

Miller

Work Unit: 0601152N.MR00001.001-0063 - Evaluation of the influence of superantigens and polyclonal B-cell activators in periodontal disease. To continue work on this IR project and bring it to closure by September, 1995. The major focus will be on the flow cytometry and rt-PCR procedures and on stimulator characterization. We have in operation a new two-dimensional gel electrophoresis system and hope to utilize it for characterization of superantigens.



Work Unit: 0601152N.MR00001.001-0063 - IL-1 production by polymorphonuclear leukocytes resident in periradicular lesions. To continue development of an *in-situ* hybridization procedure to detect cytokine mRNA. We have recently acquired a Perkin Elmer System 1000 *in situ* PCR machine and anticipate that this will aid in our efforts.

Work Unit: 0601152N.MR00001.001-0063 - Long term frozen storage of lymphocytes. Bench work has been completed and the final manuscripts need to be completed.

Work Unit 63706N.M0095.006-3014 - Influence of growth factors on gingival and periodontal ligament fibroblasts. A main goal will be to complete writing of the manuscript.

To initiate a new program designed to study lymphocyte heterogeneity in periradicular tissue. We anticipate beginning these studies in June. We continue to await approval from SRC and from the human use committee.

Solanki

- Continue to work on 2-D gel system and be better informed about the process and applications of 2-D electrophoresis.
- Eventually we would like to separate complex proteins of bacterial components.
- Start *in-situ* hybridization techniques.



IV. NMRI TOX/DET, Dayton, OH

TOXICOLOGICAL STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Briggs

Dr. Briggs is the General Manager for the NMRI Contract at the Naval Medical Research Institute/Toxicology Detachment. In this capacity he functions as the Senior Contractor Representative, as a toxicologist, and coordinator for compliance issues. It is the responsibility of this position to collaborate resources and perform research tasked by the OIC. in accomplishing the mission of NMRI/TD.

Bowen, Kimmel, Reboulet

The inhalation team was involved in three major areas of research this reporting period.

- Operant behavioral inhalation exposures.
- Spectrex Fire Extinguishant (SFE) characterization.
- Inhalation exposure chamber analysis.

Smith

Dr. Smith functions as a NMRI/TD scientist conducting research in the areas of toxicology, anatomy and physiology, chemistry and biochemistry. These duties include writing protocols for animal studies, standard operating procedures, and preparing draft and final reports for technical and peer reviewed publications. He is currently study director for several Halon alternatives projects; investigating their toxic effects as well as characterizing their individual chemical and physical properties. As study director, he is in charge of directing research which includes scheduling, budgeting, and organizing personnel and resources. He also conducts research in the areas of chemistry and biochemistry via method development of new and refinement of existing assays for toxicology screening procedures.

Prues

Ms. Prues objective for the past quarter has been to assist LT James Lindsey Ph.D. and Eldon Smith Ph.D. in research efforts ongoing at the NMRI/TD.



TMPP Project - The chemical Trimethylolpropane Phosphate is an organophosphate that is generated from the combustion of phosphate ester-based lubricants which are common aboard Navy vessels. The probability of exposure to this highly toxic substance as the result of contamination resulting from a shipboard fire is considered likely.

In a study performed by the Neurobehavioral workgroup at the institute TMPP was determined to induce epileptiform seizures. Its exact mechanism of action is undetermined.

SFE project - The Navy is currently studying the toxicity of a new fire extinguishing agent to replace the currently used Halon 1301. Halon 1301 is an ozone depleting product used to flood the sealed compartments of ships during fires. Due to regulations regarding ozone depleting substances testing for substitute products is being conducted. Aerosol agents of various formulations of the Spectrex Fire Extinguishant (SFE) are being studied for their potential inhalation toxicity to Navy personnel.

Narayanan, T.K., Jung

During the past quarter, we have continued our research in the areas of:

- Isolation and identification of the metabolites of DBNP
- Identification the TMPP receptor in the brain
- Study the toxicological effects of substances on an *in-vitro* cell model system
- Detection of amounts of neurotransmitters found in the brain (done in conjunction with LCDR John Rossi III, Lt. James Lindsey, and Ms. Sue Pries).

Ritchie

Dr. Ritchie assist in all areas of research design, protocol preparation, research supervision, statistical analysis and preparation of scientific papers and abstracts in the area of Neurobehavioral Research at NMRI/TD and the military Tri-Service Toxicology Consortium at WPAFB, OH. During the current quarter, he continued research in five areas: (a) development and validation of a comprehensive Neurobehavioral Toxicity Assessment Battery (The NMRI/TD NTAB); (b) neurobehavioral effects of exposure to low concentrations of Ozone Depleting Substance Replacements (ODSR) of military interest; (c) neurobehavioral effects of exposure to low concentrations of single and mixed combustion products; (d) anatomical disposition and effects of trimethylolpropane phosphate (TMPP), (e) development of an animal model to simulate exposures encountered by Persian Gulf War



veterans. The total budget for these work units (1408, 1409, 1420 and 1516) is \$405,000 per year.

Martin

Ms. Martin edits all technical reports, manuscripts, proposals, standard operating procedures and other materials for presentation from NMRI/TD's staff (and Tri-Service Toxicology per tasking). She ensures these materials are cleared through appropriate military channels and also serves as a liaison to procurement personnel in acquiring manuscript reprints. She ensures these publications and presentations are documented properly for reference purposes, and maintains and secures research files, graphics files and briefing files. She also lends her editorial skills as a representative of NMRI/TD in the Tri-Service Toxicology Program Development Committee. Ms. Martin also ensures that authors are provided with appropriate guidelines and instructions for formatting their publications. She assists Geo-Center's Senior Contract Representative in administrative matters. She is also responsible for developing and expanding the technical communication program at NMRI/TD and Tri-Service Toxicology under the direction of the Officer-in-Charge of NMRI/TD.

Ademujohn

Provides technical support to various aspects of ongoing projects in neurobehavioral research. During this quarter, he has been involved in testing the effects of Ozone-Depleting Substances (ODS) refrigerants, specifically, Freon -12 (Dichlorodifluoromethane), Halon 1211 and R134-a on animal models via computer-aided qualitative and quantitative methods. He also supervises animal training protocols for the modified Wahmann chamber inhalation studies and roto-wheel studies.

Connolly

Responsible for cataloging print and non-print materials for circulation, establishing circulation policies and practices, ordering and maintaining the serials collection, handling reference questions, providing interlibrary loan assistance, locating needed materials in other local libraries, and responding to requests for reprints.

Caldwell

Position Description: Department Manager/Senior Scientist III. As Department Manager, Dr. Caldwell manages GEO-CENTERS personnel working on Army and Air Force projects



for the Tri-Service Toxicology Consortium. As senior scientist, he facilitates Tri-Service Toxicology project development, develops and implements state-of-the-art risk assessment techniques for environmental and occupational hazards, and serves as the subject matter expert for: combustion toxicology, health and environmental effects of explosives and propellants, and Persian Gulf Veterans health effects. In addition, he functions as the senior GEO-CENTERS scientist in Dayton, Ohio.

Walsh

Position Description: Site Manager/Scientist III. Ms. Walsh supports the GEO-CENTERS' mission as a member of the Hazard Assessment Technical Area Group. Ms. Walsh is involved in a variety of projects including combustion toxicology, munitions and propellants, and tools development research. As Site Manager, she coordinates the research efforts of Geo-Centers employees working on Air Force and Army projects.

Geiss

Position Description: Group Supervisor I/Scientist II. As group administrator for the Pharmacodynamics (PD) Technical Area Group, Tri-Service Toxicology Consortium, Mr. Geiss has continued the development of methods and protocols for the molecular biology laboratory and ensured that all project leaders receive PD support. Experiments are ongoing for the 60-day trichloroethylene study and the hyperbaric oxygen study.

McDougal

Position Description: Senior Scientist II. Dr. McDougal is principal investigator on an Air Force Office of Scientific Research (AFOSR) Grant entitled "Species Differences in Skin Penetration". Senior scientist accomplishing Tri-Service Toxicology customer-oriented projects in the areas of dermal absorption, biologically-based mathematical modeling, and risk assessment.

Dong

Position Description: Senior Scientist I. Dr. Dong works on the "Species Differences in Skin Penetration" project. She is in charge of laboratory research to determine the rates of penetration of three model chemicals in three animal species.



Grabau

Position Description: Senior Scientist I. Mr. Grabau plans and conducts image analysis and neuropathology research on toxicology programs. He is responsible for computer images of skin cross-sections to determine the absorption effects of various toxins on animal tissues.

Narayanan, L.

Position Description: Scientist II. Ms. Narayanan is involved in the following research projects: (1) The estimation of triiodothyronine (T3), reverse triiodothyronine (rT3), thyroglobulin (hTg), and thyroid stimulating hormone (TSH) using radioimmunoassay (RIA) in trifluoriodomethane (CF₃I) and ammonium perchlorate exposed rats. (2) Estimation of progesterone, estradiol, and prolactin in ammonium dinitramide (ADN) exposed rats using RIA.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Briggs

The primary objectives were to keep existing research projects on schedule, explore ways to increase productivity, and identify opportunities for growth. Dr. Briggs continued to function with the NMRI/TD staff during the transition period. He has provided research and support services as tasked by the OIC and has helped to keep current projects in compliance with technical standards and on schedule. These activities are detailed in the next section of this report.

Bowen, Kimmel, Reboulet

There were six major objectives for this reporting period.

- Continued research with SFE.
- Completion of the Progression Ratio Strain operant behavioral inhalation exposures of test animals exposed to the ozone depleting substance replacement 1,1,1,2 tetrafluoroethane (R-134a).
- Completion of the Progression Ratio Strain operant behavioral inhalation exposures of test animals exposed to the ozone depleting substance dichlorodifluoromethane (Freon-12).



- Continued inhalation exposure chamber characterization and analysis.
- Preparation for Society of Toxicology (SOT) poster presentation (Baltimore, MD).
- Preparation for Halon Options Technical Working Conference (Albuquerque, NM).

Smith

- Write protocols to investigate the potential toxicity of SFE Formulations C and D
- Conducted the following protocols:

Protocol 1:

Range finding and multiple dose study for SFE Formulation A

Protocol 2:

Evaluation of blood gas, blood pH, hemoglobin, bicarbonate and glucose levels after exposure to the pyrolyzed by-products of SFE formulation A

Protocol 3:

Evaluation of edema in Fisher 344 Male rats exposed to by-products of pyrolyzed SFE formulation A

- Finish characterization studies of chemical and physical properties for the aerosol associated after the pyrolyzation of SFE Formulation A.
- Write final reports on SFE formulation A pilot studies.
- Present the data at national conferences and meetings.
- Develop and/or refine biochemical assays for toxicological screens.

Prues

TMPP Project - Characterize the mechanism of action of TMPP using *in-vivo* microdialysis. Timed samples of the neurotransmitters found in normal rat brain are obtained. Following determination of a normal baseline concentration for these substances via HPLC and electrochemical detection, an i.p. injection of a low dose of TMPP is delivered to the rat and changes are then observed.

SFE Project - The Navy is considering replacing it's current aerosol fire extinguishing vehicle Halon 1301, since it has been found to be damaging to the ozone layer of the earth's atmosphere.



Continued Research and Experimentation on new formulations of the SFE are being conducted. Current analysis techniques are being modified so as to expedite data analysis.

Narayanan, T.K., Jung

The objectives for this period were to:

- Continue the characterization of the TMPP receptor in the brain
- Isolate the metabolites of DBNP that are found in the urine and feces by chromatography
- Determine if hepatocytes could be used to prepare the metabolite of DBNP directly
- Go on culturing liver cells (WB344) to be used for the cell model experiment
- Measure growth curve of the hepatocytes using a non-radioactive cell proliferation assay
- Ascertain the optimal conditions for the digestion of the cells by trypsin
- Prepare brain samples for neurotransmitter analysis

Ritchie

(Work Unit 1) TMPP Mechanisms of Action: Development of Neurobehavioral Molecularization Techniques

Chemical Kindling Studies - Contract research at Bowling Green State University (BGSU) during this period replicated a previously reported result indicating that bi-daily administration of four sub-convulsive doses of TMPP (0.20 mg/kg) sensitizes rats to a subsequent amphetamine challenge, as measured by increase in locomotion activity, 30-60 days following the TMPP administration. This result clearly indicates that TMPP induces long term CNS sensitization in rats.

TMPP Deposition and Clearance - The Kennedy-Krieger Institute in Baltimore, MD was contracted to complete sophisticated autoradiography studies with TMPP. Groups of Fischer-344 rats were injected with TMPP at sub-convulsive dose levels, then euthanized at various timepoints from 5 min. To 24 hours. Analysis of TMPP binding at GABA_A, GABA_B and NMDA receptors will be conducted at each timepoint. Results of the study are expected by July 1995, and may verify previous TMPP deposition and ligand binding results.

TMPP Ligand Binding - Dr. T.K. Narayanan has reported significant progress in completing TMPP ligand binding studies in the rat. While he previously reporting no significant binding



of radiolabeled TMPP to known cholinergic, muscarinic or GABA_B CNS receptors, recent results indicate:

- TMPP-induced agonism of bicuculline binding to the GABA_A receptor in rat cerebellar membrane preparation.
- Mild displacement of radiolabeled TMPP by cold GABA in the rat cerebellar membrane preparation.

Ongoing studies are investigating competitive binding by TMPP at the benzodiazepine receptor of the GABA complex, as well as investigation of the possibility that TMPP may antagonize the formation of GABA by the decarboxylation of L-glutamic acid.

In-Vivo Electrochemistry - Dr. James Lindsey is currently preparing to begin a series of studies, utilizing fast-scan cyclic voltammetry, to evaluate neurotransmitters/metabolites in selected brain areas following TMPP administration.

Microdialysis - LT James Lindsey (Ph.D.), MSC, USN has completed development, assembly and validation of a complex system allowing simultaneous on-line Microdialysis-HPLC, recording of EEG, and video recording of changes in subject behavior. Twenty rats have been implanted with microdialysis cannulas in the nucleus accumbens; 10 were additionally implanted with cortical EEG electrodes. In the initial study, Dr. Lindsey injected rats i.p. with either TMPP or pentylenetetrazol (PTZ) and then measured changes in three transmitter and three major metabolite levels in the nucleus accumbens (area thought to be involved in seizure induction or recruitment). In a second study, Dr. Lindsey simultaneously recorded behavioral seizure activity, EEG abnormalities and correlative changes in transmitter/metabolite levels following TMPP i.p. administration. Changes in neurotransmitter/metabolite levels and return to baseline were observed. In a third study, Dr. Lindsey directly microinfused TMPP into the nucleus accumbens, and recorded convulsive behaviors such as activated sniffing, forequarter jerking and stereotypical burrowing that were not accompanied by changes in the electrocorticogram. Plans to develop fast-scan *in vivo* voltammetry with 3D-electrochemical detection in free-moving rats, flow injection analysis (FIA) and sequential flow injection analysis (SIA) are being developed.

Neurobehavioral Teratology - Protocols are being written to complete, at BGSU, the neurobehavioral teratology study outlined in the Work Unit proposal. Pregnant dams will be injected twice a day with TMPP throughout the 21-day pregnancy. Offspring will be tested for long-term CNS sensitization and other TMPP-induced effects at 90-100 days of age. The protocol is complete and awaiting ACUC approval at BGSU.



In-Vitro Neurophysiology/Neuropharmacology - A cell culture laboratory has been successfully established, using hepatocytes. Equipment and technology is now available for culturing of derived neural and neoplastic cell lines.

In-Vivo Neurophysiology/Neuropharmacology - Dr. Jingyan (Jan) Lin, M.D., Ph.D., a newly hired neurophysiologist joined NMRI/TD on 22 May 1995. All required equipment for her studies has been procured. A protocol has been written and is awaiting approval for a two year project investigating the capacity of TMPP to sensitize or induce chemical kindling in nine major brain pathways. Dr. Lin is expected to develop comprehensive multi-unit and single cell (extracellular and intracellular) recording capacity at NMRI/TD.

Other Neurobehavioral Studies - Attention Deficits: A series of studies have been completed at NMRI/TD demonstrating that administration of sub-convulsive doses of TMPP (0.20 mg/kg, but not 0.10 mg/kg) reliably disrupted a well trained operant habit in rats. This disruption was further shown to be temporally consistent with the appearance of i.p. injected TMPP in the brain, and with the observation of paroxysms in the EEG. This study has developed a new method for determining the onset of neurobehavioral effects induced by an injectable drug or toxicant.

Place Preference: Preliminary research at BGSU indicates that previous TMPP administration may induce a place preference in rats. This would infer that exposure to sub-convulsive doses of TMPP may be rewarding in rats.

Timidity Testing: Research at BGSU indicates that low doses of TMPP reduced exploratory behavior in rats and induced "timidity" as measured in an elevated plus-maze.

Learning and Recall: Research at BGSU indicates that sub-convulsive doses of TMPP were shown to increase latency to find food in 4 baited arms of an eight-arm radial maze, compared to controls. On a subsequent test two hours later, however, TMPP treated rats outperformed controls in finding food now in the four arms of the radial maze that were not previously visited. This implies that TMPP may reduce memory consolidation or recall.

Play (Social Behavior): Research at BGSU indicates conclusively that a single administration of a low dose of TMPP significantly reduces play behavior in juvenile rat pairs, as measured by dorsal contacts and play pins. The effect, however, exhibited tolerance within three days.



(Work Unit 2) Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (1408)

(Work Unit 3) Non-Lethal Measures of Toxicity: Performance Decrements (1409)

(Work Unit 4) Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (1420)

Experimental Evaluation of Performance Endpoints (1408, 1409, 1420)

Experiments on Neurological Activity:

- Research completed.
- A paper "The NMRI/TD Behavioral Seizure Identification Scale" is awaiting final editing and internal approval for submittal to *Epilepsia*. [1408, 1409, 1420]. This paper presents a new method for qualifying and quantifying all behavioral seizure activities associated with animal responses to toxic exposures.

Measures of Motor System Integrity and Endurance:

- The journal article "The NMRI/TD Roto-Wheel: A new apparatus for multiple measures of physical incapacitation" [1408, 1409, 1420] is awaiting internal clearance for submittal to Behavior Research Methods, Instruments, & Computers. The article provides a technical description of the construction and applications of the NMRI/TD Roto-Wheel.
- The journal article "Incapacitation and recovery from brief exposure to oxone-depleting substance replacement HFC-134a [1408, 1409] is awaiting internal clearance for submittal. This article details use of the NMRI/TD Roto-wheel to measure toxicant-induced deficits in motor system integrity, equilibrium and endurance.
- All NMRI/TD Roto-Wheel testing of rats, examining incapacitation and recovery from brief exposure to carbon monoxide (CO) [1409, 14200] is complete. Correlative carboxyhemoglobin blood gas analysis is also complete.

Measures of Physiological Irritation:

- Rating scales for sensory and respiratory system irritancy have been reviewed and adapted from utilization with gas/vapor exposures. The emotionality rating system



originally developed by King and Meyer has been adapted for rating of subject emotionality [1408, 1409, 1420].

- All exposures to date (Freon-12, R-134a, Halon-1211 and CO) have failed to produce any physiological irritancy detectable by gross observation or necropsy analysis [1408, 1409, 420].

Measures of Sensory Acuity, Including Color Vision:

- Eleven white Carneaux pigeons have completed extensive operant training in preparation for ODS/non-ODSR and fire gas exposures. Pigeons have been trained and delayed matching-to-sample spectral and geometric pattern discrimination tasks [1408, 1409, 1420]. Exposure to Freon-12 is scheduled to commence on 1 June 1995.

Measures of Social Factors & Emotionality:

- A study has been completed at Bowling Green State University, Department of Psychology evaluating changes in play behavior (dorsal contacts, pins, etc.) between pairs of juvenile rats during toxic administrations. This research was presented in two scientific posters.
- The play (social) behavior analysis system, used commonly at BGSU has been adapted for use at NMRI/TD. An open field apparatus has been built for housing within a Hinners-type chamber [1408, 1409, 1420]. Software has been written for data collection and analysis. Preliminary training of experimenters in scoring of social/play behaviors is complete. A required photo-beam platform for automated measurement of total locomotion behavior has been ordered.

Measures of Motivational Level & Frustration

- A progressive ratio operant task has been developed and tested with rats, clearly differentiating motivational deficits associated with toxic exposures from motor system and higher cognitive deficits [1408, 1409, 1420].
- A schedule-induced polydipsia task has been developed and is currently being tested to evaluate frustration effects in rats during toxic administrations [1408, 1409, 1420].
- Forced swim test (FST) tanks are currently being constructed (100 gallon Plexiglas tanks) for use with rats in evaluation of endurance versus motivational level during toxic exposures [1408, 1409, 1420].



- A Porsolt forced swim test apparatus has been developed for use in evaluating rat depression versus motivation during toxic exposures [1408, 1409, 1420].

Measures of Spatial & Temporal Discrimination

- A Morris water maze (MWM) is currently being developed for analysis of toxicant-induced deficits in spatial discrimination [1408, 1409, 1420].
- A unique operant two-choice discrimination task has been developed and extensively tested for evaluation of temporal discrimination deficits in rats [1408, 1409, 1420].
- A differential reinforcement of low rates of responding (DRL) task is being developed to further evaluate temporal discrimination deficits in rats and pigeons [1408, 1409, 1420].

Measures of Higher Cognitive Decrement

- A protocol for the operant training of 100 pigeons was submitted to the Animal Care and Use Committee on 24 December 1994 is now approved. This protocol will allow development and testing of several operant measures of high cognitive deficits [1408, 1409, 1420].
- A journal article Neurobehavioral toxicity of brief exposure to ozone-depleting substance replacement R-134a as measured by operant performance has been written and is awaiting internal clearance for journal submission [1408, 1409, 1420].

Transition to 6.3, Prog. 8 Tri-Service (1408, 1409, 1420) - A book chapter, two journal review articles and two platform presentations concerning development and application of the NTAB and neuro-molecular batteries have been completed [1408, 1409, 1420].

(Work Unit 5) Persian Gulf War (PGW) Simulation Using Sprague-Dawley Rats (U.S. Army and NMRI/TD)

An outline for development of a simulation of toxicant exposures encountered by Persian Gulf War veterans was completed.

Seven neurobehavioral tests were selected to evaluate exposed rats, and appropriate equipment was prepared or procured.

A protocol for experimentation was written and submitted to the ACUC.



In coordination with Bill Sontagg, Bill Binole, Harry Leahy, Rich Marker and M.J. Walsh exposure chambers, rat housing cages and shock delivery equipment is being procured or built.

Animal exposures are planned to begin 1 July 1995.

Martin

Ms. Martin's objectives for this quarter were to continue to perform the duties outlined in the afore-mentioned position description. Other objectives include presenting posters at and preparing other materials for the Navy Environmental Health Center's Workshop on Preventive Medicine and Occupational Health and Tri-Service Toxicology's April Conference. Ms. Martin also finalized preparations for marketing at various other conferences such as the Society of Toxicology Conference in Baltimore Maryland and the Air Force Single Manager's Conference at Wright-Patterson AFB. Also, Ms. Martin's continuing objective of this quarter has been to edit, reformat, and recreate an informational package geared toward reservists considering NMRI/TD for their active duty assignment. This package includes information on the history of Tri-Service Toxicology, descriptions of technical areas, and project descriptions. In addition, this package will include many graphics and photos generated, scanned, and imported into the text in-house. Also, Ms. Martin began working on a multimedia presentation for the Tri-Service Toxicology Consortium and conducted an analysis of the Tri-Service Toxicology Internet servers. Another of Ms. Martin's objectives was to assist NMRI/TD's principal investigators with the preparation of new and continuing work unit summaries for submission to NMRI. Ms. Martin's final objective was to assist the Administrative Officer of NMRI/TD in compiling a NMRI/TD-specific informational package for submission to the new Commanding Officer of the Naval Medical Research Institute.

Binole, Rix

The objectives for this period were: (1) Upgrade of main servers to NTAS 3.5 (2) Conversion of library and old main server to NTAS 3.5. (3) Install SMTP server dameon for NTAS to free us from the Air Force VAX. (4) Expand internet capability by adding WAIS databases. (4) Provide technical support for in house staff.

Ademujohn

The major technical objectives for this quarter are as follows:



- Testing various ODS substances on animal models using diminished capacity as the endpoint in Wistar rats.
- Range finding using roto-wheel-trained animals and measuring different stages of diminished capacity.
- To compile, catalog and computerize the above mentioned data.
- To train pigeons for problem solving protocols.
- Daily maintenance of pigeon intake and logging performance results.
- To obtain operant testing and training data for animals used in R134-a Ratio Straining exposure testing.
- To organize, catalog and generate computer graphics, cumulatively from the above mentioned data.
- To maintain data for future reference in upcoming publications.
- To be responsible for the procurement of all materials used in testing and training protocols.
- Responsible for documenting and maintaining operant weights.
- Responsible for writing standard operating procedures for pigeon training protocols.
- Responsible for making daily accurate and detailed entries and updates of all work unit laboratory books.
- Responsible for compiling information for and conducting weekly meetings with/between work unit P.I.'s and laboratory technicians.

Connolly

- Catalog materials not yet cataloged.
- Maintain the library.
- Update the database containing card catalog to facilitate searching on the internet.
- Provide library service to the toxicology community at WPAFB.

Caldwell

Pre-/pos-timplantation loss in ammonium dinitramide (ADN) treated rats - Analyze data from the *in vivo* study and close out this section of the project.

Combustion toxicity of advanced composite materials - Prepare a draft technical report on the results of the experiments conducted with B-2 ACM. Transition the F-22 combustion project to another investigator.



Vapor phase lubricants - Analyze data from experiments in which rats were exposed to a blend of 85% PAO/15% t-butylphenyl phosphate in an acute inhalation toxicity study of this candidate vapor phase lubricant. Assist in preparation of draft technical report of results.

Persian Gulf Veterans research - Prepare and submit an animal use protocol to investigate the effects of exposure to multiple chemical stressors coupled with an induced psychological stress.

Ammonium Perchlorate - Continue to provide scientific support to the Armstrong Laboratory Toxicology Division and assist in developing a Cooperative Research & Development Agreement (CRDA) which is currently being negotiated by the USAF. Prepare and submit an animal use protocol for the toxicity study.

Walsh

Inhalation Toxicity of Tricresyl Phosphate Vapor Phase Lubricant - Continue screening of four vapor phase lubricants. The lubricants are structurally similar to Tricresyl Phosphate, which is known to have a toxic ortho isomer and may form other toxic phosphate compounds. These new compounds appear to be less toxic since they do not produce the ortho isomer; however, they are being evaluated for potential phosphate compound formation under use conditions to establish safe exposure levels for humans.

In Vitro-In Vivo Extrapolation - Continue work on the tools development program to establish reliable techniques for a rapid *in vitro* screen to test for rank-orders toxicity and the detection of subtle biochemical changes in primary hepatocytes exposed to toxic compounds.

Primary Hepatocyte Isolation - Continue to meet an increasing demand for primary hepatocytes used within the Tri-Service Toxicology Division. One example of the increased demand is a current study goal to establish hepatocyte metabolism experiments to provide data on the realistic metabolic rates observed *in vivo*. Trichloroethylene (TCE) was chosen because it has been the subject of many whole-animal (rat) exposures in Tri-Service. Much is known about its metabolism and rate of uptake by the rat. Further, human subjects will be exposed to TCE in a study sponsored by the U.S. Army next quarter in North Carolina. Prior work which assessed the metabolism of other compounds by isolated hepatocytes has yielded data which accurately predicted the metabolism observed in the whole animal. The intent of the current study is to establish this system using isolated hepatocytes of the rat and human to develop a predictive tool for the metabolism of TCE and other compounds.



Geiss

Identify needs in molecular biology research support and design a technical approach to fulfill those needs. This includes developing protocols and research methods for evaluation of biological effects of Air Force-related materials.

Cooperate in current research relating to the toxicological effects of trichloroethylene (TCE), its metabolites and other compounds.

Perform follow-on analysis of gerbil brain RNA samples from an experiment involving hyperbaric oxygen (HBO) treatment.

Train other scientists in molecular biology research methods.

McDougal

Continue collecting pharmacokinetic information for 3rd chemical and 3rd animal species for AFOSR grant.

Bring "Skin Absorption Time" (a quantitative method of relaying the potential risk of exposure to chemicals in the workplace) to a public forum for discussion and criticism.

Determine feasibility of setting up *in vitro* skin diffusion cells to estimate systemic hazards from dermal exposure to Unicharge (Modular Artillery Charge System), its components and other military chemicals.

Dong

To improve dermal exposure methods and analytical techniques to meet the need of the $C_6F_{13}I$ dermal penetration study and to conduct $C_6F_{13}I$ dermal exposure studies in F-344 rats.

To develop a workable dermal exposure method for the Hairless guinea pig in a skin penetration study.



Grabau

Species Differences in Skin Penetration - Initiate evaluation and development of image analysis algorithms to quantify immuno-histochemical markers of early events in chemically-induced skin inflammation.

Combustion Toxicity of Advanced Composite Materials (ACM) - Further evaluate results from the recently completed ACM combustion studies and plan follow-up *in-vivo* experiments.

Trichloroethylene (TCE) Biologically Based Health Risk Model - Conduct further image analysis to quantify subcellular (PCNA, P450 isoenzyme and other markers) changes in liver cells exposed to TCE.

Intra-Laboratory Research (ILR) Grant - Hepatic Apoptosis in Mice and Rats - Develop and provide image analysis techniques on tissue collected from 100 rats. Develop and prepare to conduct image analysis on tissue from 200 mice to study apoptosis that will be available in July 1995.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Briggs

- Helped to re-write and implement the 5454 notification for organizational function.
- Assured the completion of all taskings made by the OIC.
- Assisted with planning and resource deployment for project support. This included the addition of a research technician position which was filled.
- Assisted with the program development functions and contributed to the planning and costing of two major research activities that will likely result in additional income for the Institute.
- Actively participated in the Interagency Agreement with the EPA and DOE in establishing priority chemicals to be evaluated for toxicity and risk assessment. Attended three meetings to assist with the planning and coordination of resources for continuing this project.



- Prepared and submitted a quality management program and a reproductive system risk assessment plan as tasked.
- Assisted in performing risk assessments as tasked.
- Monitored and assisted in the planning of toxicology profile studies relating to HFC 236fa, the Navy refrigerant candidate in the Ozone Depleting Substance replacement project. This included a Quality Assurance Audit, study data reviews, and the planning and coordination of additional metabolism studies which will be useful in planning for future studies.
- Attended the Society of Toxicology Meeting in Baltimore, MD for continuing education.
- Presented a platform presentation at the Conference on Risk Assessment Issues for Sensitive Human Populations, April, WPAFB.

Bowen, Kimmel, Reboulet

A Spectrex analysis system was built around a 0.7 m³ inhalation exposure chamber in laboratory 120 of Bldg 433, NMRI/TD. Data from both 50 g/m³ and 80 g/m³ characterization experiments of SFE Formulation A were collected. Corresponding data collected in February 1995 at Naval Research Laboratory (Chesapeake, MD) in their 56 m³ fire testing facility was assimilated. The data collected at both sites was assimilated for comparative analogy.

The results from the data analysis were presented at the Halon Options Technical Working Conference (Albuquerque, NM) May 9-11.

Results from inhalation toxicity exposures of test animals to atmospheres of pyrotechnically generated SFE aerosols were presented at the March 1995 Society of Toxicology annual conference held in Baltimore, MD.

Inhalation toxicity exposures of test animals to the ozone depleting substance replacement R-134a were completed for the Neurobehavioral Group. A group of test animals was repeatedly exposed to atmospheres of R-134a while undergoing a progressive ratio strain operant tasking. A progressive ratio strain test (PR-3) is one in which the test animal presses the feed lever a given amount of times. After each reward (a sugar pellet), the increment is increased by three and that many bar presses must be accomplished prior to receiving the next reward. The first exposure concentration level was 4% (40,000 ppm) and the test animals did not show a performance decrement until a level of 12% (120,000 ppm) was reached.



Inhalation toxicity exposures of test animals to the ozone depleting substance Freon-12 were completed for the Neurobehavioral Group. A group of test animals was repeatedly exposed to atmospheres of Freon-12 while undergoing a progressive ratio strain operant tasking. The initial exposure concentration level was 4% and test animals did not show a performance decrement until a level of 16% was reached. Inhalation exposure chamber atmospheres were monitored using a Miran 1ACVF-A.

The modified Hinner's type inhalation exposure chamber #2 was prepared for a temporal distribution experiment. A sample probe was placed in five points within an operant testing box while inside the exposure chamber. A target concentration of R-134a was generated and monitored using a pre-calibrated Miran 1ACVF-A infra-red spectrophotometer. The highest temporal variation observed was 2.11%. The results achieved were satisfactory and showed stable generation methods with little temporal variation. Figure 5 is a graphical representation of the data collected from real-time chamber concentration analysis.

Smith

- Presented a poster at the Society of Toxicology 1995 Annual Meeting held in Baltimore, MD on March 5-9, 1995.
- Presented a platform presentation at the Halon Options Technical Working Conference held in Albuquerque, NM on May 9-11, 1995.
- Began a blood gas study.
- Analyzed data from field study at the Chesapeake Beach Detachment of the Naval Research Laboratory. The study examined the physical properties of SFE Formulation A in a full scale environment.
- Developed and refined biochemical assays for the microplate. These assays include acid phosphatase, alkaline phosphatase and total protein.

Prues

TMPP Project (Dr. John Rossi) - Electroencephalogram studies of rats being used by Dr. Lindsey are being conducted while simultaneously video taping the rats both before and after injection with TMPP. For this study the following procedures are performed:

- Surgical placement of EEG electrodes.
- Video taping of rat behaviors before and after dosing with TMPP.
- Data analysis reading of EEG videos, marking seizure patterns for time and duration and then correlating the behavioral changes with microdialysis events.



- Importing the EEG data into a spreadsheet to analyze frequencies.
- *In vitro* HPLC determination of neurotransmitters in rat and chicken brains from a study being conducted by Dr. John Rossi III at Bowling Green University. For this study, the following procedures have been performed; preparation of samples, loading and processing samples through HPLC and detector, data analysis, and processing of data to determine changes in neurotransmitter levels through the use of specialized software.

TMPP Project (Dr. James Lindsey) - *In vivo* on line microdialysis studies of the effects of TMPP on rat brain neurotransmitters involves efforts in the following methods: surgical implantation of microdialysis probe guides into the target region of the brain of Sprague Dawley rats, standardization of microdialysis probes, preparation of mobile phase and solutions of neurotransmitters for determination of standard curves, and data analysis

TMPP Project (Dr. Eldon Smith) - A receptor binding study of TMPP was commissioned by Dr. Eldon Smith while he was in charge of the TMPP funding. The method involves using an immunofluorescent technique to study the (alpha and beta) GABA receptors and the MMDA receptors in the brains of Fisher 344 Rats in an attempt to characterize the neuroconvulsant properties of this chemical. The study is being carried out in collaboration with Dr. Michael Johnston of the Kennedy - Krieger Institute in Baltimore, Maryland. The study involved: weighing and separating Fisher 344 rats into groups, timed injections of rats with various concentrations of TMPP, and assisting in the timed sacrifices

SFE Project - Study methods to determine the toxic effects of inhalation exposure to the fire extinguishing agent SFE include scaling down of commercially available assay methods for use with a microplate reader. The procedures include assays for: ACP, ALP, Protein determination (Pierce BCA kit), data analysis, and ordering and organizing laboratory equipment and supplies.

Narayanan, T.K., Jung

TMPP binding to the GABA receptor in both the cerebellum and cortex were studied using equilibrium dialysis. The protein was added to a dialysis tube that contained 3 ml of buffer. The experimental samples were done the same way except for the addition of 0.1 M TMPP to the buffer solution. The tubes were stoppered and placed in a rotator overnight and radioactivity of the protein solution inside the dialysis tubing and of the buffer surrounding was measured. It was found for both the cerebellum and cortex that TMPP does not have a



high affinity for the GABA receptor. Therefore, this does not appear to be the site of TMPP binding.

DBNP Project - The radioactive peak was isolated from the urine and feces by HPLC. The fractions from repeated runs were collected, pooled, and then lyophilized. The *in-vitro* metabolism of DBNP by hepatocytes was compared to the *in-vivo* metabolism. Some radioactivity was detected in the water fraction of the sample but none was found in the control (3 ml of Gibco BRL media with 2.28 mg of ^{14}C -DBNP). The methanol fraction of both control and the experimental samples eluted with the same retention time as the standard DBNP. This clearly shows there is no alteration of the parent compound by the constituents of the RPMI 1640 media itself. However, the presence of radioactivity in the water fraction indicates that some of the DBNP was modified by the cells. The water portion when run on the HPLC was too dilute to detect. Therefore, repetitive samples are being collected and pooled. Once a sufficient number of counts has been collected, the sample will be run on the HPLC again.

Cell Model - The growth rate was measured using a Non-Radioactive Cell Proliferation Assay from Promega. Cells from a confluent plate were taken and dispersed among new plates (35 x 10 mm) at varying concentrations which was determined by a cell count performed manually on a hemacytometer. The plates were incubated for 24 hours in a 5% CO_2 atmosphere and at 37°C. The media was then removed from the plate and replaced with a fresh 1 ml of serum. The solutions from the Promega kit were mixed (50 ml of PMS/ 1 ml of MTS) in the dark. The reaction solution [200 ml] was added to each plate and, after incubation, the absorbency at 490 nm was taken. Incubation times of 1, 2, 3, and 4 hours were tried to learn which gave the best results and it was found that the full 4 hour incubation gave the maximum absorbance without depleting the reagents.

Several concentrations and exposure times of trypsin in PBS-EDTA were used for the dislodgment of live cells from the culture plate. We were trying to ascertain the optimal conditions that would release the cells from the plate without causing clumping and still have viable cells. We found that a 10X solution of trypsin-EDTA that digests for 10 minutes provides the best results. There is little clumping of the cells and most are viable.

Neurotransmitter Study - Chicken brain samples consisting of four different brain sections (diencephalon, striatum, brain stem, and forebrain) with experimental and control samples were processed. The samples were first frozen in liquid nitrogen and then weighed. They were placed in 2 ml of 0.17 N HClO_4 and homogenized by Polytron. This was followed by



centrifugation at 20,000 rpm for 20 minutes. The supernatant was measured and stored at -80°C for analysis by Lt. Lindsey and Ms. Sue Prues.

Martin

In addition to editing a multitude of manuscripts, presentations, work units, abstracts, technical reports, standard operating procedures, and galley proofs, Ms. Martin has accomplishments in other areas as well. She completed the reservist informational package in a timely fashion and presented a poster at the NEHC Conference. She also ensured that the Tri-Service Toxicology Consortiums information booth was erected and functional at the April conference in addition to co-presenting two poster presentations. Her greatest accomplishment this quarter was the creating, designing, formatting, reproducing, and distributing of an instructional manual on toxicology-related Internet use on behalf of Tri-Service Toxicology. Nearly two hundred of these manuals were created and distributed at Tri-Service Toxicology's April Conference and were in even greater demand. In addition, many requests for copies of Ms. Martin's "Functional and Collaborative Efforts of Tri-Service Toxicology" poster were submitted and responded to. Ms. Martin's on-line analysis of Tri-Service Toxicology's Internet servers spawned a Tri-Service effort to improve and modify the current Internet servers.

Binole, Rix

- Expansion of our internet presence via poster display at Hope Hotel Toxicology seminar.
- Allow access to our library holdings via internet access.
- Completed upgrade of main servers to NTAS 3.5.
- Restructured LAN systems WAN access per WPAFB directives.
- Install of SMTP server is on hold while we wait to be enrolled in a beta test program. This will allow us to receive the SMTP server software for free when it is released.
- Conversion of library server is on hold pending arrival of new computer systems. The old main server will be removed and either taken out of service or converted to NTAS 3.5 and used as a dedicated internet server thus freeing the Lab LAN server from this burden.
- Completed development of inhalation test program.
- Completed development of new neuro behavioral test program involving Self Induced Polydipsia.
- Procurement of all software/hardware for use at NMRI/TD.
- Technical support for all software/hardware installed.



Ademujohn

- Compiled, organized, and cataloged via computer-aided graphics, the weekly data on Fixed and Progressive Ratio Straining (FR & PRE3) protocols.
- Catalogued and analyzed incoming weekly data on R-134 a (4%, 8%, 12%) cumulative data from FR and PR response curves.
- Catalogued and compiled all incoming FR and PR weekly data on Freon-12 exposures, (4%, 8%, 12%, 16%), respectively.
- Implemented an SOP on the procedures for pigeon "Match" protocol training.
- Maintenance of all laboratory work unit notebooks.
- Implemented several data methods to compile training data and weight maintenance of the pigeon operants.
- Modified pigeon operant boxes for uniform problem solving display.
- Implemented an effective operant box disinfection program.
- Responsible for procurement of gases used for testing.

Connolly

- A complete inventory of the library was conducted to identify lost, strayed or stolen books. Inventory results showed a 1.5% shrinkage over 3 years. Acceptable shrinkage, under library standards, is anything under 3% per year.
- 90 books cataloged and prepared for circulation.
- 20 articles obtained from local libraries.
- 133 articles entered into the reprint database and file.
- 6 interlibrary loans obtained.
- 6 books obtained from local libraries to fulfill requests.
- 34 article requests filled from this library's collection.
- 3 literature searches conducted using in-house CD-ROM database capabilities.
- 2 people trained in new search techniques for CD-ROM searching.
- 1 library instruction covering all aspects of library functions written.
- 16 reference questions answered.
- 78 telephone inquiries on journal locations in local area handled successfully.
- 1925 records in the card catalog database updated to facilitate searching, including looking up and entering CAS numbers where appropriate.
- Attended supply training held here by NMRI.
- Attended "Conflict Resolution for Women" training in the local area.
- Continued as part of the IPT on Libraries and Information Centers.
- Attended Conference on Risk Assessment Issues for Sensitive Human Populations.



- Sorted through files of old articles written here and discarded those too old to be in demand.
- Toured other base libraries as part of IPT.

Caldwell

Pre-/post-implantation loss in ammonium dinitramide (ADN) treated rats- Data from the *in vivo* study were analyzed and uterine samples were harvested for morphological review and analysis by electron paramagnetic resonance (EPR). ADN was shown to prevent implantation of the embryo in the uterus. An animal use protocol to elucidate the preimplantation effects has been submitted. The project will be transferred to another investigator to conduct this phase of the study.

Combustion toxicity of advanced composite materials - An Armstrong Laboratory technical report on the results of the experiments conducted with ACM used on the B-2 bomber is being prepared. Animal exposures to determine acute inhalation toxicity will be performed during the next quarter for ACM used on various aircraft. The F-22 combustion project has been transferred to Ms. Merry Jane Walsh.

Vapor phase lubricants - Rats were exposed to a blend of 85% PAO/15% t-butylphenyl phosphate in an acute inhalation toxicity test of this candidate vapor phase lubricant. Data are currently being analyzed and will be incorporated into an Armstrong Laboratory technical report being prepared for the Wright Laboratory sponsor (Project leader is Ms. Merry Jane Walsh).

Persian Gulf Veterans research - An animal use protocol has been submitted to the local ACUC for review to investigate the effects of exposure to multiple chemical stressors coupled with an induced psychological stress. Dr. Caldwell was invited to attend a special meeting of the Research Working Group of the Persian Gulf Veterans Coordinating Board to discuss ongoing funded research projects which will be held 14-15 June in Washington, D.C.

Ammonium Perchlorate - The Perchlorate Study Group met at Aerojet, Inc., Sacramento, CA on 18 April 1995. Dr. Caldwell presented a research proposal for toxicity work to be conducted at the Tri-Service Toxicology Consortium, Wright-Patterson AFB through a Cooperative Research & Development Agreement (CRDA) funding mechanism currently being negotiated by the USAF. An animal use protocol for the toxicity study was submitted to the local ACUC. I attended the U.S. Environmental Protection Agency (Environmental



Criteria and Assessment Office, Cincinnati) Cooperative Agreement research review on 25 April 1995. The threshold model developed at Virginia Commonwealth University under an EPA cooperative agreement will be used to model data derived from the ammonium perchlorate study. Submitted a paper entitled, "Study Design for the Toxicity Evaluation of Ammonium Perchlorate" to the Joint Army-Navy-NASA-Air Force (JANNAF) Interagency Propulsion Committee for presentation at the July, 1995 Environmentally Sound Processing Technology Workshop to be held in San Diego, CA.

Risk Assessment - Chaired the Risk Assessment Methodology Working Group of the SERDP funded Environmental Risk Assessment Project meeting on 24 April. This meeting brought to closure initial efforts conducted under the still to be ratified charter and set the research agenda for future efforts under this project.

Walsh

Inhalation Toxicity of Tricresyl Phosphate Vapor Phase Lubricant - Two additional compounds were tested. The first was a chemical supplied by FMC "Durad 620B" Tert-Butyl-Phenyl-Phosphate. The second was supplied by Mobil which was comprised of 15% Durad 620B & 85% 2cSt PAO. Results of these exposures revealed Neurotoxic Esterase (NTE) activity results similar to the natural and synthetic TCP chemicals tested last quarter. The animals were exposed to a four hour inhalation exposure to the Durad 620B, showing 34.7% NTE activity, with the Durad 125 "Synthetic TCP" results showing 49.31% activity, as well as the Ashland Natural TCP having a 43.5% activity. The animals exposed to the mixture of 620B and PAO all expired within the first two hours of the four hour exposure. Pathologic evaluations were performed on these animals and revealed significant edema surrounding large vessels and major airways. The technical report titled "Inhalation Toxicity of Vapor Phase Tricresyl Phosphate", has been submitted to the client in the draft form. Ms. Walsh is the first author with two GEO-CENTERS co-authors (D. J. Caldwell and L. Narayanan). An abstract for the JANNAF December, 1995 Subcommittee Joint Meeting under the same title was also submitted.

In vitro-In vivo Extrapolation - During this period liver perfusions for primary hepatocyte isolation used to develop various assays were performed. Ms. Walsh contributed to the validation of eight assays. Three assays remain to be validated for an *in vitro* test system. This data will be used as a cellular toxicity factor which accounts for the probability that given a particular cellular concentration of the active form of the toxicant a particular cellular response will occur.



Primary Hepatocyte Isolation - During this quarter Ms. Walsh performed liver perfusions for primary hepatocyte isolation in support of various metabolism studies utilizing TCE. Human hepatocytes are also used in this study, provided by a tissue bank. She was responsible for the validation of the viability and total count of these cells.

Geiss

Hybridization assays were performed on samples from the 60-day study with probes made from plasmid templates for ICAM-1, GCS-LC, and GCS-HC. Additional template stocks have been prepared for TGF-b1, PKC-a, ODC, and IL-1a.

Literature searching and computer analysis of nucleic acids was performed to determine what hybridization parameters were needed and what results were to be expected to confirm our analysis of specific mRNA targets. New DNA analysis software was acquired to help with this task. A portion of Mr. Geiss's time has been spent in familiarization with this software.

Mr. Geiss has worked up a manual method for the *in situ* hybridization assay of mRNA in paraffin-embedded tissue sections. This will be used for the localization of target mRNA species in liver tissue.

He also continues to spend a few hours a week training technicians in molecular biology techniques. The training is achieved in conjunction with actual experimental work.

Since the last reporting period Mr. Geiss has assumed new duties relative to the laboratory administrative structure. As the Group Administrator for the Pharmacodynamics (PD) group, he is responsible for resource allocation, training and professional development of the 13 people in that group. He also interacts with project leaders to help identify their technical needs and provide solutions for them.

Mr. Geiss attended a conference on molecular and genetic toxicity. This meeting provided information that is useful in the development of assays for the evaluation of this type of toxicity. These assays may be implemented into the battery of tests already in use in our lab.

McDougal

Presented "Skin Absorption Time" approach at the American Industrial Hygiene Association Conference in May 1995.



Submitted first publication detailing the relationship between chemical damage to the skin and change in rate of chemical penetration to "Microscopy Research and Techniques."

Dong

The $C_6F_{13}I$ dermal study has been improved by increasing the size of skin area exposed and especially by improving the analytical sensitivity.

Confirmed that the blood concentration of $C_6F_{13}I$ in rats resulting from dermal exposure is extremely low over a 4-hour exposure period.

A "serial trial and error" technique has been applied to hairless guinea pigs to overcome the difficulties of keeping the exposure cells on their skin.

Grabau

Species Differences in Skin Penetration - A poster presentation (first author) was made at the 34th Annual Meeting of the Society of Toxicology detailing image analysis methods for evaluating epidermal thickness in dermal toxicology studies. A second poster, "Dermal Absorption Kinetics of Liquid Chloropentafluorobenzene (CPF_B) and 1,2-Dichlorobenzene (DCB)" was co-authored and presented. A journal article, "Inflammatory Damage To Skin By Prolonged Contact With 1,2-Dichlorobenzene and Chloropentafluorobenzene", has been accepted for publication in "Microscopy Research and Techniques." Immuno-histochemical reagents to quantify early events in chemically-induced skin inflammation have been received and development of staining procedures is on going.

Combustion Toxicity of Advanced Composite Materials (ACM) - Image analysis of images obtained from twenty one separate combustion experiments of ACM in a modified UPITT II combustion chamber was completed. Posters (second author) describing the methods and results of this research program were presented at the 34th Annual Meeting of the Society of Toxicology (March 1995) and the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995).

Trichloroethylene (TCE) Biologically Based Health Risk Model - Image analysis methodologies to quantify subcellular (PCNA) changes in liver cells exposed to TCE and research results were presented as posters (second author) at the Society of Toxicology Annual Meeting (March 1995) and at the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995). Image analysis methodologies were conducted



to quantify subcellular (PCNA) changes in liver cells resulting from a multi-week exposed to TCE. Research results are in draft for publication.

Hepatic Apoptosis in Mice and Rats - There have not been any new developments in this research program. The start of this project has been delayed.

Dr. Grabau attended the LABCAT users group meeting (Orlando, FL 19-23 March 1995) to evaluate the suitability of the software for animal toxicity studies.

Image analysis support been provided to develop a new animal model to evaluate reproductive toxicity. An application and abstract for a poster presentation has been submitted to the International Society for the Study of Xenobiotics for the fall 1995 meeting.

Narayanan, L.

In this quarter research has focused on three following areas:

- Measurement of hTg and rT3 and T3 levels in control and CF₃I exposed rats, using RIA;
- Measurement of TSH in control and CF₃I exposed rats, using RIA.; and
- Measurement of progesterone, estradiol, and prolactin in control and Ammonium dinitramide(ADN) exposed rats during pre- and post-implantation, using RIA.

Significant differences were detected in exposed animals as compared to controls for both chemicals evaluated.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Briggs

- Plan and initiate the HFC 236fa reproductive study in a non-rodent species.
- Review the data from the completed toxicology studies on HFC 236fa and assist with the final report preparation.
- Review the preliminary metabolism study data and assist with the decision relating to whether a lifetime bioassay study in rodents is required. Additional planing for the toxicology profile will be required if no bioassay will be required. This will require extensive planning by the project team.



- Initiate the reproductive system evaluation studies and associated quantitative risk assessment investigation for DBNP.
- Follow up on the respiratory distribution study possibility and coordinate resources if the project is funded.
- Continue to perform tasks as assigned by the OIC in accomplishing the mission of NMRI/TD.
- Prepare a position paper relating to GLP compliance.

Bowen, Kimmel, Reboulet

- Continuation of SFE aerosol characterization.
- Continuation of SFE inhalation toxicity studies.
- Continuation of inhalation exposure chamber temporal and spatial distribution analysis.
- Initiation operant inhalation toxicity exposures of pigeons to Freon-12.

Smith

- Complete characterization studies on SFE formulation A.
- Begin characterization studies on SFE Formulation C and D.
- Begin development of microassays for b-glucuronidase and lactate dehydrogenase.
- Submit final reports on SFE Formulation A pilot studies for publication.
- Begin research on developing a new screening procedure for determination of cardiac sensitization.

Prues

Continue to assist in the research being conducted at the NMRI/TD providing my technical expertise as required. For the upcoming quarter work with Dr. James Lindsey on his TMPP research as well as assisting Dr. Eldon Smith in preparative work for the upcoming SFE project.

Naryanan, T.K., Jung

Objectives for the next reporting period are to increase our productivity in the lab and continue the studies on separating and analyzing the metabolite(s) of DBNP, the TMPP binding studies, and work on the cell model and neurotransmitter project.



Ritchie

(Work Unit 1) TMPP Mechanisms of Action: Development of Neurobehavioral Molecularization Techniques

- Completion of TMPP/operant studies.
- Initiate TMPP behavioral teratology studies at BGSU.
- Completion of microdialysis studies with nucleus accumbens and commencement of studies with inferior colliculus, substantia nigra, etc.
- Commencement of TMPP studies using FIA, fast-scan cyclic voltammetry and multi-unit stimulation/recording.

(Work Unit 2) Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors

- Completion of rat operant studies with Freon-12 versus R-134a.
- Commencement of pigeon operant studies with Freon-12 versus R-134a.
- Procurement of Halon-1301 and R-227ea for rat and pigeon studies.

(Work Unit 3) Non-Lethal Measures of Toxicity: Performance Decrements

- Commencement of rats studies evaluating play behavior, Porsolt Forced Swim Test, and Morris Water Maze.
- Completion of Schedule-Induced Polydipsia tests with rats.
- Completion of goals and objectives for Work Unit 1409 that closes 10/95.

(Work Unit 4) Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures.

- Development of mixed combustion gas (5 gas) delivery system, incorporating Perkin Elmer FTIR, computer driven valves, etc.
- Testing of CO2 toxicity with rats (NMRI/TD Roto-Wheel).

Ademujohn

- To accurately and efficiently compile, log, organize, and analyze all incoming data from inhalation studies.
- To train rodents for various testing protocols.



- To train pigeons for upcoming testing protocols.
- To maintain a clean and orderly laboratory environment.
- To provide technical assistance in modified Wahmann chamber studies.
- To provide technical support in testing relative toxicity of R134a, Halon 1301.
- To provide technical support to operant chamber analysis of rat exposures to low concentrations of R134a, Halon 1301.
- To provide technical support in streamlining operant training methods for upcoming pigeon and rodent training protocols/testing, i.e., in play behavior, swim testing and pharmacological studies.
- To procure and document pigeon maintenance pertaining to preparatory requirements for 'shaping' activities, pre-testing and testing protocols.

Martin

Ms. Martin's objectives for next quarter are to continue editing manuscripts, reports, standard operating procedures and presentations at NMRI/TD and the Tri-Service Toxicology Consortium as tasked. She will also update the publication files and ensure all publications are documented by NMRI/TD's parent commands. Ms. Martin will continue working on the Tri-Service Toxicology multimedia presentation and represent Tri-Service Toxicology at the Navy Pollution Prevention Conference in Washington D.C. and the International Society of Toxicology Conference in Seattle, Washington. She will continue to develop the technical communication program at NMRI/TD under the direction of the OIC and work closely with Tri-Service counterparts to ensure alignment with interservice processes and procedures. She will continue to provide GEO-CENTERS' Senior Contract Representative as needed and will work with GEO-CENTERS' ADP staff in updating and improving the Tri-Service Toxicology Internet Servers.

Binole, Rix

In the next quarter ADP will continue to implement those software and hardware products which increase automation and productivity. Projects scheduled for the coming quarter include:

- Continue to add to and improve our network capabilities.
- Continue to evangelize our internet capabilities to the toxicology community.
- Complete conversion of Library server to NTAS 3.5.
- Install SMTP server dameon (5) Improve and add additional WAIS databases.
- Begin to convert all outdated databases to Foxpro.



- Continue to provide technical support for TOXDET personnel.
- Develop support software where needed.

Connolly

- Continue updating card catalog files.
- Continue cataloging.
- Continue providing service to the toxicology community at WPAFB.
- Continue as member of base level IPT on Libraries and Information Centers.

Caldwell

Combustion toxicity of advanced composite materials - Finalize technical report on the results of the experiments conducted with B-2 ACM. Provide senior scientific guidance to the F-22 combustion project (Ms. Walsh is project leader). Initiate animal exposures to determine acute inhalation toxicity of ACM used on various aircraft.

Vapor phase lubricants - Finalize technical report of results of rat exposures to a blend of 85% PAO/15% t-butylphenyl phosphate in an acute inhalation toxicity test of this candidate vapor phase lubricant.

Persian Gulf Veterans research - Revise, if necessary, animal use protocol to reflect current thinking on potential causes of "Persian Gulf Veterans Illnesses" and devise experimental approach to address these potential causes.

Ammonium Perchlorate - Continue to provide scientific support to the Armstrong Laboratory Toxicology Division and assist in developing a Cooperative Research & Development Agreement (CRDA). Revise, if necessary, the animal use protocol for the toxicity study. Present paper, "Study Design for the Toxicity Evaluation of Ammonium Perchlorate" to the Joint Army-Navy-NASA-Air Force (JANNAF) Interagency Propulsion Committee at the July, 1995 Environmentally Sound Processing Technology Workshop to be held in San Diego, CA.

Methodology Working Group. Develop and implement state-of-the-art risk assessment methodologies as results from ongoing experimental protocols become available.



Walsh

Inhalation Toxicity of Tricresyl Phosphate Vapor Phase Lubricant - The final technical report is expected to be submitted during next quarter. Additional funding is being requested for further study of a chosen compound which was initially tested under this study.

Combustion Toxicity of Advanced Composite Materials - Function as a project leader of the F22 project titled "Combustion Toxicity of F-22 Advanced Composite Material." This study is expected to be started next quarter.

In vitro - *In vivo* Extrapolation - Perform additional liver perfusions as well as contribute laboratory support to the validation of three additional assays under the *in-vitro* test system project.

Primary Hepatocyte Isolation - Continue to support this study by providing primary hepatocytes, processing and validating the human hepatocytes.

Geiss

To continue analysis of tissue samples from the 60-day TCE study with histone, glyceraldehyde-3-phosphate dehydrogenase, and gamma-glutamylcysteine synthetase probes.

Develop assays for other probes of interest, such as protein kinase C-alpha and ornithine decarboxylase.

Design and synthesize oligonucleotides for use in analysis of mRNAs in samples from other projects.

Perform additional experiments for the hyperbaric oxygen project.

Carry out tasks required as PD Group Administrator.

McDougal

Continue collecting pharmacokinetic information for 3rd chemical and 3rd species for AFOSR grant.

Submit AFOSR proposal for new three-year project.



Set up and test *in vitro* skin diffusion cells using Unicharge and its components and develop mathematical models to estimate permeability constants and flux.

Dong

To continue developing the exposure technique in the Hairless guinea pig model for use in dermal penetration studies.

To continue $C_6F_{13}I$ dermal studies on F-344 rat and Hartley guinea pigs.

Grabau

Species Differences in Skin Penetration: Further development of methods to study early events of inflammation is planned.

Combustion Toxicity of Advanced Composite Materials (ACM): Initial statistical analysis and modeling of present ACM combustion data is planned. Planning for future *in-vitro* and *in-vivo* combustion studies is expected.

Trichloroethylene (TCE) Biologically Based Health Risk Model: Additional immuno-histochemical markers are under evaluation to study the toxic effects of TCE and will require image analysis.

Hepatic Apoptosis in Mice and Rats: The future status of this research program is uncertain.

Image Analysis Development: Further training in image analysis methods and techniques is planned to take place at the North Carolina State University, Raleigh, NC, 27-29 June 1995.

Narayanan, L.

Radiolabelling thyroid stimulating hormone antigen with iodine (^{125}I) using the chloramine-T method and estimation of thyroid stimulating hormone levels in control and CF_3I exposed rats.

Estimation of neurotoxic esterase activity in control and ammonium dinitramide exposed rats.



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Continuation of the ongoing research projects by repeating some of the experiments and maintaining involvement in research projects, such as ammonium perchlorate, that are of interest to Air Force and Army.



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